Sudan University of Science and Technology College of Animal production science and Technology Department of Fisheries and wild life Science

EFFECT OF REPLACING FISFMEAL WITH ALKOARA SILAGE IN THE FORMULATION OF DIETS FOR NILE TILAPIA *(O.niloticus).*

إستبدال مسحوق مخلفات السمك بسيلاج سمكة الكواره في تركيب عليقة اسماك البلطي النيلي.*(O.niloticus)* وأثره في النمو

A Thesis Submitted in Partial Fulfillment of the Requirement of the

B.Sc.Degree in Fisheries and Wild life Science (Honor)

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October, 2016

Privation:

بسم الله الرحمن الرحيم

(وقل ربي زدني علماً)

صدق الله العظيم

سورة طه الآية (114)

ABSTRACT:

The experiment was carried out a period of 45 days; from19/6 to 3/ 8/ 2016.

The experiment design flow through system was developed at the fish hatchery department of fisheries science and wildlife, to determine the effect of replacing fishmeal with alkoara silage in the formulation of diets for Nile tilapia (*O.niloticus*). 240 Nile tilapia fingerlings (average weight 6.40 gm.) Fish were distributed in flow through system of 12 plastic aquariums (34*22*18 cm) each containing about 40 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 20 fingerlings / aquarium. The experiment included 4 treatments with 3 replicated aquariums for each. Feeds C_0, T_1, T_2 and T_3 (The diets replacing 0, 25, 50 and 75% of fish meal protein content by fish silage.Results indicated that final body weight (BW), weight gain (WG) and specific growth rate (SGR) of *O. niloticus* increased with increasing level of fish silage in diets. WG was found 7.90, 7.01 and 7.98 for T1, T2 and T3 respectively. The data were analyzed by one-way analysis of variance (ANOVA, F test) and LSD for significantly different means at a significance level of 0.05 using SPSS version 19.

Keywords: *Growth performance, Diets, treatment, AKoaraSilage, O.niloticus*

المستخلص

أجريت هذه الدراسه لمعرفة أثر إستبدال مسحوق مخلفات السمك بسيلاج مصنوع من سمك لكواره في عليقة أسماك البلطي النيلي وتحديد اثره على معدل نمو أسماك البلطي لمدة 45يوم في الفتره من 19\6 الى 3\8\2016.

صممت هذة التجربه بنظام الماء التدفق المستمر وركب في مفرخ الأسماك التابع لقسم علوم الاسماك والحياة البرية

استعملت لتجربة مئتان وأربعون من زريعة أسماك بلطي النيلي بمتوسط وزن بلغ 6.40 جرام

قسمت الأسماك بشكل عشوائي في احواض بلاستيكية بلغ عددها 12 حوض بالأبعاد التالية (34*22*18) سم ووضع في كل حوض 20 سمكة وكانت سعة كل حوض 40 لتروتمت أقلمة الاسماك لمدة ثلاثة أيام قبل البدء. قسمت التجربة إلى أربعة متغيرات (المتغيرT3, T1,T2 ,C0)

حيث C0 تمثل التجربة الضابطة بلا اضافة وT1 التجربة المضاف اليها نسبة 25% سيلاج وتمثل T2 و التجربة المضاف اليها نسبة 50% سيلاج والتجربة T3 تمثل المضاف اليها 75% سيلاج .

أظهرت الدراسة ان الوزن المكتسب وجد كالاتي : 7.90بالنسة لتجربة T1 و7.01 بالنسة لتجربة T2 وكانت افضل النتائج التجربة T3 حيث سجلت اعلى وزن مكتسب 7.98

و أستخدم لتحليل البيانات إحصائيا برنامج SPSS version 19 واستخدم اختبار (ANOVA, F test بمستوى معنوية 0.05

كلمات مفتاحية : معدل نمو ، عليقة ،تجربة ،سيلاج السمك ، *O.niloticus*

DEDICATION

To my dear parents
To my teatcher's
To my brother's and my sister's
To my family ...
To my friends

To my dear parents
To my teatcher's
To my brother's and my sister's
To my family ...
To my friends

ACKNOWLEDGMENTS:

Ole Thanksgiving and the end of God Almighty.

Then after him all tired of me

(family particularly my father Kamal

, my mother Samia

and my sister Susan.

After them, thank all of my teacher's ,

specifically including: D.Asaad Hassan Wdaa D. Sarah Bushra Ustaz Fawzi and Ustaz Motaz and all of the work of the Department of Fish and Wildlife Sciences.

Thank greats Sudan University of Science and Technology

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CHAPTER ONE

INTRODUCTION

Aquaculture is the farming of aquatic organisms: fish, mollusks, crustaceans, aquatic plants, crocodiles, alligators, turtles, and amphibians. Farming implies some form 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. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resource, with or without appropriate licenses, are the harvest of capture fisheries**. FAO (2009-2016)**

The world fish production in 2000 was 130.4 million tons of fish, out of which 94.8 million tons were from fishing and 35.6 million corresponded to aquaculture production. From 1996 to 2000, aquaculture increased from 25.7 to 35.6 million metric tons. The aquaculture production for 2001 was estimated as 37.5 million metric tons. In 1997, 122 million metric tons of fish were produced in the world, which represented US$ 49 billion in revenues. (**FAO, 2004).** The Brazilian production of fish was 798,719 metric tons, out of which 115,398 tons came from the national aquaculture production, with consequent revenue of US$ 198,000,200 (**Ostrensky *et al*.; 2000**).

Tilapia are the third most important cultured fish group in the world, after carps and salmonids, Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4% during 1970–2002. They are widely cultured in about 100 countries in the tropical and subtropical regions.

As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of total farmed finfish in **2002 (El-Sayed A.M (2002).**

Fish meal remains the most expensive ingredient in aquaculture feeds and fish farmers now seek its replacement with less expensive alternative protein feedstuffs. One of such alternatives is fish silage prepared from whole fish; fishery wastes by-products or fish farm mortalities **(Lo *et al.*, 1993)**. Fish silage is prepared either by mineral and/or organic acid preservation (acid silage) or by anaerobic microbial fermentation (fermented silage). The latter is preferred in developing countries because it is cheaper to produce, involves simple artisanal technology which is adaptable at cottage level and possesses good storage properties **(Dong et al., 1993).**

Objectives:

a) To determine the effect of replacing fishmeal with alkoara silage in the formulation of diets for Nile tilapia (*O.niloticus*).
b) To effect of alkoara silage in water quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture

Fish is a vital source of food for people. It is man's most important single source of high-quality protein, providing ~16% of the animal protein consumed by the world's population, according to the **Food and Agriculture Organisation (FAO) of the United Nations (1997)**. It is a particularly important protein source in regions where livestock is relatively scarce—fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China. The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein **(FAO, 2000).**

Fish also has substantial social and economic importance. The FAO estimates the value of fish traded internationally to be US$ 51 billion per annum **(FAO, 2000)**. Over 36 million people are employed directly through fishing and aquaculture, and as many as 200 million people derive direct and indirect income from fish **(Garcia and Newton, 1997)**. Consumption of food fish is increasing, having risen from 40 million tonnes in 1970 to 86 million tonnes in 1998 **(FAO, 2000**), and is expected to reach 110 million tonnes by 2010 **(FAO, 1999).**

2.2 Tilapia Biology

2.2.1 Taxonomy of tilapia

Species: Oreochromis niloticus **(Linnaeus 1758)**

Family: Cichlidae

Order: Perciformes

Class: Actinopterygii

The Nile tilapia Oreochromis niloticus is a deep-bodied fish with cycloid scales. Silver in colour with olive/grey/black body bars, the Nile tilapia often flushes red

during the breeding season **(Picker & Griffiths 2011)**. It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) **(FAO 2012).** The average size (total length) of O. niloticus is 20 cm **(Bwanika et al. 2004).**

2.3 Natural distribution and habitat

O. niloticus is native to central and North Africa and the Middle East **(Boyd 2004).** 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).**

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).**

2.4 Environmental requirements

Tilapia can tolerate a wider range of environmental conditions—including factors such as salinity, dissolved oxygen, temperature, pH, and ammonia levels than most cultured freshwater fishes can. In general, most tilapia are highly tolerant of saline waters, although salinity tolerance differs among species. Nile tilapia is thought to be the least adaptable to marked changes (direct transfer, 18 parts per thousand in salinity); Mozambique, blue, and redbelly (T. zilli) are the most salt tolerant **(El-Sayed 2006)**. With the exception of Nile tilapia, other tilapia species can grow and reproduce at salinity concentrations of up to 36 parts per thousand, but optimal performance measures (reproduction and growth) are attained at salinities up to 19 parts per thousand **(El-Sayed 2006)**. Tilapia are, in general, highly tolerant of low dissolved oxygen concentration, even down to 0.1

mg/L **(Magid and Babiker, 1975),** but optimum growth is obtained at concentrations greater than 3 mg/L **(Ross, 2000).**

Temperature is a major metabolic modifier in these fish. Optimal growing temperatures are typically between 22° C (72° F) and 29° C (84° F); spawning normally occurs at temperatures greater than 22° C (72° F). Most tilapia species are unable to survive at temperatures below 10° C (50° F), and growth is poor below 20° C (68° F). Blue tilapia are the most cold tolerant, surviving at temperatures as low as 8° C (46° F), while other species can tolerate temperatures as high as 42° C (108° F; **(Sarig, 1969; Morgan, 1972; Caulton, 1982; Mires, 1995).**

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

2.5 Nutritional requirements

2.5.1 Protein

Fish do not have a specific requirement for crude protein (CP) per se, but rather they need a combination of essential amino acids. Therefore, the profile of dietary protein is important when formulating diets for tilapia. Dietary proteins are used continuously by fish for maintenance,growth, and reproduction functions. When fed in excess, protein may be used as energy; however, the latter function is not desirable because of the expensive cost of proteins. The protein requirement of tilapia decreases with age and size (table 2), with higher dietary CP concentrations

required for fry (30–56%) and juvenile (30–40%) tilapia but lower protein levels (28–30%) for larger tilapia **(Winfree and Stickney, 1981; Jauncey, 1982; Al Hafedh, 1999; Siddiqui et al., 1988; Twibell and Brown, 1998).**

2.5.2 Lipids

Dietary lipids provide a major source of energy, facilitate the absorption of fat soluble vitamins, play an important role in membrane structure and function, serve as precursors for steroid hormones and prostaglandins, and serve as metabolizable sources of essential fatty acids. **Winfree and Stickney (1981)** found that for tilapia up to 2.5 g, the optimum dietary lipid concentration was 5.2%, decreasing to 4.4% for fish up to 7.5 g. **Jauncey (2000)** suggested that to maximize protein utilization, dietary fat concentration should be between 8 and 12% for tilapia up to 25 g, and 6 to 8% for larger fish. As with most fish, tilapia appear to have a requirement for n-6 (linoleic) fatty acids, and to a lesser extent, a requirement for n-3 (linolenic) fatty acids. Dietary lipids should supply at least 1% of n-6 fatty acids **(Teshima et al., 1982).**

2.5.3 Carbohydrates

Fish do not have a specific requirement for carbohydrates, because amino acid and fatty acid precursors can supply the required glucose via gluconeogenesis. This does not imply that carbohydrates should not be included in tilapia diets, however. Carbohydrates provide a relatively inexpensive source of energy compared to protein, and their inclusion can improve the quality of pelleted feeds. Tilapia can effectively utilize carbohydrate levels up to 30 to 40% in the diet, which is considerably more than most cultured fish **(Anderson et al., 1984; Teshima et al., 1985).**

The relationship between concentrations of dietary protein and energy is important in fish nutrition. Diets should be balanced to maximize the use of protein for growth by providing optimal amounts of energy as carbohydrates and lipids.

The ratio of protein to energy (P:E; mg/ Kcal) varies with fish age and size. For tilapia the optimum ratio for growth varies between 68 and 125, depending on species and size **(Winfree and Stickney, 1981; Shiau andHuang, 1990).**

Scientific classification of Alkoaara fish

Kingdom: Animalia

PHylum: Chordata

Class: Actinopterygii

Order: Characiformes

Superfamily: Alestioidea

Family: Alestidae

Genus: Alestes

J. P. Müller & Troschel, 1846

2.6 Use of fish waste

According to Food and Agriculture Organization **(FAO, 2004)**, the world fish production in 2000 was 130.4 million tons of fish, out of which 94.8 million tons were from fishing and 35.6 million corresponded to aquaculture production. From 1996 to 2000, aquaculture increased from 25.7 to 35.6 million metric tons. The aquaculture production for 2001 was estimated as 37.5 million metric tons.

In 1997, 122 million metric tons of fish were produced in the world, which represented US$ 49 billion in revenues. The Brazilian production of fish was 798,719 metric tons, out of which 115,398 tons came from the national aquaculture production, with consequent revenue of US$ 198,000,**200 (Ostrensky et al.; 2000).**

The world production of aquatic organisms from aquaculture increased from 28.82 in 1997 to 30.86 million metric tons in 1998. In the same period, there was a

reduction of over 7 million metric tons in the production of captured aquatic organisms **(FAO, 2000)**.Sixty percent of the fish captured worldwide are used in the fresh fish market or processed as frozen, canned or cured foods, generating a considerable amount of waste material. The volume of waste produced by processing plants is calculated to be about 50% of the total processed fish. To that, we can add a considerable amount of fishing produce that is considered inadequate for human consumption due to its low commercial value, as well as the amounts discarded and refused by Federal/State Inspections in fresh commercialization markets **(Rebeca *et al*.; 1991)**.Thus about 50% of the world fish production becomes waste material, which means an expressive amount of 65.2 million metric tons of fish waste **(Ferraz de Arruda, 2004).**

The largest contributor to the increase in fish production is aquaculture, which is one of the fastest growing agricultural activities in the world.

According to **Ostrensky et al. (2000),** the Brazilian aquacultural production increased from 23,390 metric tons in 1991 to 115,398 metric tons in 1998, an increase of 393%, growing about 26% a year, going up from the 35th position in 1991 to the 26th position in 1997 in the ranking established by FAO.

2.7 Fish silage

Fish silage is a liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic-acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish **(FAO, 2003).**

Acid silage was developed in 1920 by A. I. Virtanen, using hydrochloric and sulphuric acid for the conservation of forages. Experiments with fish began in

Sweden in 1936, using hydrochloric, sulphuric, and formic acids and sugars **(Tatterson and Windsor, 1974).**

Organic acids, such as formic acid, are generally more expressive than the mineral ones. However, they produce less acid silages that do not need to be neutralized before being used. The bactericide action must be considered. A mixture of formic and propionic acids has been recommended. If a 1:1 formic-propionic ratio is used as well as the addition of 3% volume/weight to the biomass, the silage obtained is stable, with an acidified aroma **(Kompiang, 1981)**. The use of formic acid for the preservation of wasted material to be used in rations began after World War II. In the preparation of chemical silage, the choice of preservation reagents is made rom inorganic acid, a mixture of acids, organic acids or the mixture of organic and inorganic acids, which, as formic acid, are generally more expansive than common inorganic acids, but produce silages that are not excessively acid, and, therefore, do not need neutralization before being used. Inorganic acids, such as hydrochloric acid and sulphuric acid can be recommended due to their low cost **(Oetterer, 2002).**

For the preparation of the chemical silage, the raw material must preferably be presented in small pieces or be ground. Afterwards, acid is added to allow for its action until liquefaction takes place.

Normally, room temperature is used and the storage provokes the desired biochemical modifications. It is essential that the mixture is stirred so that the raw material can be in contact with the acid, once non-treated parts of the material can putrefy. After the initial mixture, the silage process naturally begins, but occasional stirring helps in obtaining the desired uniformity **(Oetterer, 2002).**

2.8 Fish feeding

The use of fish silage in the feeding of fish has been widely studied. Due to the similarity of this protein source with the raw material and low cost, especially when compared to fish meal, silage has a high potential use in aquaculture. **(Hussain and Offer, 1987; Fagbenro et al.; 1994; Vidotti et al.; 2003; Goddard and Perret, 2005). Fagbenro et al.(1994) and Fagbenro and Jauncey (1998)** studied the nutritional value of diets containing microbial fish silage partially dehydrated by the addition of soy meal, poultry by-products, or bone and meat powder, and found no significant differences in the performance and protein use when compared to diets based on fish meal. The experiment showed that these diets, especially the ones including silage and soy meal, could be used to feed tilapias, *Oreochromis niloticus* (omnivorous), and the North African catfish, *Clarias gariepinus* (carnivorous), with no changes in its performance, use of protein and carcass composition.

In salmon farming, the search for diets that promote fast growth, favour fish sanitation, result in quality products and have low cost is essential.Some authors, trying to evaluate the use of fish silage in salmon (*Salmo salar*) feeding, concluded that although this silage did not promote a better development, it did not cause a decrease either, and its cost was much lower **(Espe et al.; 1994; Heras et al.; 1994).**

The natural food for the pink abalone (*Haliotis fulgens*) is microalgae. In the nited States, this microalgae is used as the main food. Commercial diets are produced only in Japan and New Zealand, their high cost makes them unviable. Thus,artificial diets are necessary, preferably at low cost. **Viana *et al.* (1999)** evaluated the use of silage as an alternative feed. The authors concluded that fish silage was very attractive, but not very palatable. They recommended that it should be used with a more tasteful ingredient, such as corn meal.

Carnivorous fish from the Amazon area, and concluded that they presented a higher level of ingestion. The most important was that the technology used in the elaboration of biological ensilage was adequate for the production of a product to be used as protein source in the preparation of rations for arapaima, which could be produced at artisanal level, without sopHisticated equipment or specialized laborers.

In nutritional terms, the diet given to aquacultured fish can define the fatty acids profile in the fish meat. Thus, for example, the catfish (*Ictalurus punctatus*) diet, supplemented with 2, 4, and 6% of menhaden (*Brevoortia tyrannus*) oil provided 5.7, 8.4 and 10.1% of omega-3 fatty acids, respectively, in the fish muscles. Also, the supplementation of the tilapias (*Oreochromis niloticus*) with sardine (*Sardinella, sp*) oil resulted in a larger proportion of eicosapentaenoic and docosahexanoic acids **(Haard, 1992). Lessi *et al*. (1989)** and **Ximenes Carneiro *et al.* (1996)** tested biological fish ensilage in the feeding of Black- Finned Pacus (*Colossoma macropomum)* fingerlings and shrimp post-larvae (*Macrobrachium rosembergii)* for the first time in Brazil. Ensilage was found an alternative and a potential substitute for fish meal and meat and bone meal in Black-Finned Pacus rations.

The apparent digestibility is one of the main resources in the evaluation of the potential of the ingredient to be used in fish rations. **Borghesi (2004)** determined the apparent digestibility coefficient (ADC) of the energy, nutrients and amino-acids of acid silage (AS), biological silage (BS) and enzymatic silage (ES) in Nile tilapias (*Oreochromis niloticus*) weighing on average 94.54 ± 12.66g. The values for digestible energy found were 4041.32; 3663.95 and 3394.20 Kcal/kg for AS, BS and ES, respectively. The ADC values found were: 92.01; 89.09 and 93.66% for crude protein; 89.86, 87.61 and 90.10% for ash; 82.52, 78.98 and 82.96% for dry matter; 81.72, 73.99 and 80.27% for calcium; and 77.86, 79.21 and 81.46 %

for phosphor in AS, BS and ES, respectively. The average ADC of amino acids were: 91.83; 90.76 and 94.61% for AS. BS and ES, respectively. These results show the possibility of using AS, BS and ES as protein ingredient in balanced rations for aquaculture, as partial substitute of fish meal.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The experiment was carried out a period of 60 days; from19/6 to 3/ 8/ 2016. The experiment design flow through system was developed at the fish hatchery department of fisheries science and wildlife, college of animal production science and technology, Sudan University of science and technology.

3.2 Materials:

- 12 Containers keep of liquids
- 12 PCV Pips
- 12 Plastic aquaria
- Kidz measuring water quality
- One barrel 4-inch
- Sensitive balance
- Thermometer
- Small fish nets

3.3 Experimental design*:*

Two hundred and forty Nile tilapia fingerlings (average weight 6.40 gm.) were obtained from outdoor ponds of fish hatchery.

Fish were distributed in flow through system of 12 plastic aquaria (34*22*18cm) each containing about 40 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 20 fingerlings / aquarium. The experiment included four treatments with three replicated aquariums for each. Feeds C_0, T_1, T_2 and T_3 (The diets replacing 0, 25, 50 and 75% of fish meal protein content by fish silage.

Each aquarium was equipped with a hole to remove excess water from the aquarium capacity connected with downspout. Thermostat controlled heater fixed at 28 - 30^{0}C. Fish were fed 6% of body weight daily three times (9 am, 12pm and 3 pm). About 15% of aquarium water was changed daily before morning feeding to remove the Waste digestion.Fish were weighed are measured every 10 days and feed ration was adjusted accordingly. Fish survival was monitored also during this experiment. Water quality parameters (temperature, oxygen, pH, nitrate and ammonia concentrations).

Table (3.1): Ingredients composition (%) of the experimental diet dry basis

Ingredient (%)	Experimental diets			
	control	T1	T2	T3
fish meal	40	30	20	10
fish silage	0	10	20	30
ground nut cake	20	20	20	20
surgum meal	10	10	10	10
wheat bran meal	8	8	8	8
starch	3	5	7	8
bread floor	10	10	10	10
mineral mix pre.mix	1.5	1.5	1.5	1.5
vitamin mix pre.mix	1.5	1.5	1.5	1.5

Table (3.2) proximate chemical analysis for ingredients and different environments.

Ingredients Treatment	D.M	C.P	C.F	E.E	Ash	N.F.E
Fish silage	91.37±0.55^a	25.85±0.17^c	5.65±0.07^b	23.18±0.05^c	15.16±0.02^d	**30.15±0.02^d**
Control	95.35±0.07^a	18.78±0.01^d	7.69±0.14^a	7.32±0.14^d	20.43±0.02^a	**45.77±0.07^a**
T1	94.35±0.71^b	21.46±0.02^c	5.40±0.02^c	10.57±0.04^c	16.70±0.14^b	**45.85±0.04^a**
T2	93.95±0.20^c	25.72±0.11^b	6.12±0.04^b	12.06±0.03^b	14.45±0.49^c	**41.64±0.68^b**
T3	92.13±0.04^d	30.23±0.02^a	6.20±0.02^b	13..56±0.02^a	12.17±0.04^d	**37.82±0.02^c**

^{a,b,c,d} Means in the same column with superscript are significant different at level (p<0.05). whereas:
D.M=Dry Matter. C.P=Crude Protein .C.F= Crude Fiber. E.E=Either Extrat.Ash.N.F.E=Nitrogen Free Extrat.

3.4 Water quality

Temperature, pH, dissolved oxygen (DO) and ammonia were estimated by aqua sol kits during the experimental period according to APHA (1995). PHysico-water as follows:

3.4.1. pH:

A clean test tube was filled with 5 ml of water to be tested (to the line on the tube).Five drops of high range pH Test solution were added, holding dropper bottle upside down in a completely vertical position to assure uniformity of drops. The test tube was caped and inverted tube several times to mix solution.

The test result was readied by comparing the color of the solution to the appropriate High Range pH Color Card (freshwater or Saltwater was choosed). The tube was viewed in a well- lit area against the white area of the card. The closest match indicators the pH of water sample.

3.4.2 Dissolved Oxygen:

I.D.O. Fixing: the dissolved Oxygen requires to be fixed before testing.

The **D.O**. test bottle was rinsed 2 – 3 times with sample water .and filled till it overflows with the sample water then stoppered the bottle and ensure that no air bubbles were trapped inside. Ten drops of **D.O.1**were added and were followed by 10 drops of **D.O.2**. And mixed well. Waited for a minute. A brown precipitate was formed and setted. The bottle was firmly stoppered and shacked thoroughly. The bottle was kept in a safe place for a minimum 20 minutes. Ten to twelve drops of **D.O.3**were added. And the bottle was shacked till the precipitate dissolved. More drops were added if required to dissolve the precipitate. Sample was used for tested.

Proceed for D.O. determination as described in **II**

II.D.O. determination:

Ten ml. of sample (from step 3 of **D.O**. fixing) in the test jar was taken. Four drops of D.O.4were added .and mixed well.**D.O.5**was added, counted the number of drops while mixing, until the blue color disappears.

Calculation:

Dissolved Oxygen ppm = 0.65 × [No. of drops of **D.O.5**]

3.4.3Total ammonia (NH_3/NH_4):

A clean tube was filled with 5 ml of water to be tested (to the line tube).Eight drops from Ammonia Test Solution Bottle #1were added, holding the dropper bottle upside down in a completely vertical position to assure uniform drops. Eight drops from Ammonia Test Solution Bottle #2were added, holding the bottle upside down in a completely vertical position to assure uniform drops. The test tube was caped and shacked vigorously for 5 seconds.

Five minutes were waited for the color to develop. The test result was readied by compared the color of the solution to the appropriate ammonia Color Card (the fresh water color card was used). The tube was viewed in a well – lit area against the white area of card. The closest match indicates the ppm (mg/l) of ammonia in the water sample.

3.5 Growth and feed utilization

Initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), survival rate, protein efficiency ratio (PER), protein productive value (PPV) and energy retention (ER) were measured using the following equations:

Weight gain (g) = final weight – initial weight;

Weight gain % = 100 x weight gain / initial weight;

Specific growth rate (SGR; %/day) =

100 (Ln final weight – Ln initial weight) / days;

Feed intake (g fish/day) = $\frac{total\ feed\ intake\ per\ fish}{number\ of\ days}$

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g);

Survivor Rate % = $\frac{initial\ number\ of\ fish\ stocked - mortality}{number\ of\ days} \times 100$

3.6 Proximate composition determination

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

3.6.1 Moisture Content Determination:

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

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

3.6.2 Crude Protein Determination:

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

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

Whereas:

Va = volume of HCL used in titration

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

14 = conversion factor of ammonium sulfate to nitrogen

6.25= conversion factor of nitrogen to protein

Wt= weight of sample

N= normality of NaoH

3.6.3 Crude Fat Determination:

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

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

3.6.4 Ash Content Determination:

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

$$\textbf{Ash \%} = \frac{\text{Ash weight}}{} \times 100$$

Sample weight

3.7 Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA, F test) and LSD for significantly different means at a significance level of 0.05 using SPSS version 19

CHAPTER FOUR

RESULTS

4.1 Growth and conversion variables

Growth performance parameters; initial average weight, final average weight, average weight gain, average specific growth rate (SGR), survivor rate (SR %) and feed conversion ratio (FCR) are shown in (Table 4.1) .

Proximate analysis of feeds is similarly shown (Table 4.2).

Survival rate (SR) conducted on all the feeds showed significant difference in the four week during this study (Table 4.3).

Table (4.1): mean growth performance and feed utility for *O.niloticus* fed different level of fish silage.

Treatments Parameters	Control	T1	T2	T3
Initial weight	6.42 ± 0.21^{a}	6.48 ± 0.42^{a}	6.24 ± 0.23^{a}	6.46 ± 0.42^{a}
Final weight	$12.420.63^{c}$	14.38 ± 1.39^{a}	13.26 ± 0.19^{b}	14.44 ± 1.83^{a}
WG	5.99 ± 0.54^{b}	7.90 ± 1.07^{a}	7.01 ± 0.06^{a}	7.98 ± 2.12^{a}
SGR	1.72 ± 0.08^{a}	1.98 ± 0.13^{a}	1.88 ± 0.00^{a}	1.98 ± 0.24^{a}
WG%	11.42 ± 0.63^{c}	13.39 ± 1.39^{a}	12.26 ± 0.19^{b}	13.45 ± 1.89^{a}
FCR	2.54 ± 1.96^{b}	2.89 ± 0.15^{b}	3.08 ± 0.06^{a}	2.89 ± 0.56^{b}

[a,b,c] Means in the same row with superscript are significant different at level ($p<0.05$).

Table (4.2): mean proximate chemical composition of *O.niloticus* fed with different level of fish silage.

Nutrients	D.M	C.P	E.E	Ash	N.F.E	C.F	M
Treatment							
Control	38.21±0.02^{b}	19.26 ± .02^{b}	8.22 ± .02^{a}	9.92 ± 0.02^{d}	36.49 ± .04^{c}	0.02 ± .00^{a}	61.79±0.02^{a}
T1	41.01±0.00^{d}	28.44±0.00^{c}	10.55±0.04^{c}	16.80±0.00^{b}	45.80±0.02^{a}	5.40±0.01^{c}	58.99±0.01^{a}
T2	39.68±0.01^{c}	27.80±0.00^{b}	12.09±0.00^{b}	14.75±0.07^{c}	41.14±0.02^{b}	6.21±0.08^{b}	60.32±0.01^{b}
T3	22.83±0.01^{a}	32.24±0.01^{a}	13.58±0.00^{a}	12.15±0.01^{d}	37.83±0.01^{c}	6.19±0.01^{b}	57.17±0.00^{c}

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

whereas:
D.M=Dry Matter. C.P=Crude Protein .C.F= Crude Fiber. E.E=Either Extrat.Ash.N.F.E=Nitrogen Free Extrat.M=Moisture.

.

Samples Treatments	Sample 1	Sample2	Sample3	Sample4
Control	98.33±2.88^{b}	100.0±0.00^{a}	98.33±2.88^{b}	100.0±0.00^{a}
T1	100.0±0.00^{a}	100.0±0.00^{a}	100.0±0.00^{a}	100.0±0.00^{a}
T2	100.0±0.00^{a}	100.0±0.00^{a}	100.0±0.00^{a}	100.0±0.00^{a}
T3	96.66±2.88^{c}	100.0±0.00^{a}	100.0±0.00^{a}	100.0±0.00^{a}

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

Table (4.3): SR for O.niloticus and different level of fish silage

Table(4 .4): spilling Water quality parameters a measured for experimental different level of fish silage**.**

Treatment / Parameters	T0	T1	T2	T3
PH	6.84±0.33[a]	6.67±0.26[a]	6.72±0.313[a]	6.68±0.33[a]
D.O	5.55±2.29[a]	4.06±0.33[b]	5.09±1.63[a]	5.42±1.90[a]
Temperature	29.48±1.30[a]	29.76±1.39[b]	29.44±1.82[a]	29.67±1.14[a]
NO_2	0.00±0.00[a]	0.00±0.00[a]	0.25±0.17[a]	0.25±0.28[a]
NH_3/NH_4	0.00±0.00[a]	0.00±0.00[a]	0.25±1.19[a]	0.50±023[a]
PO_4	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
NO_3	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

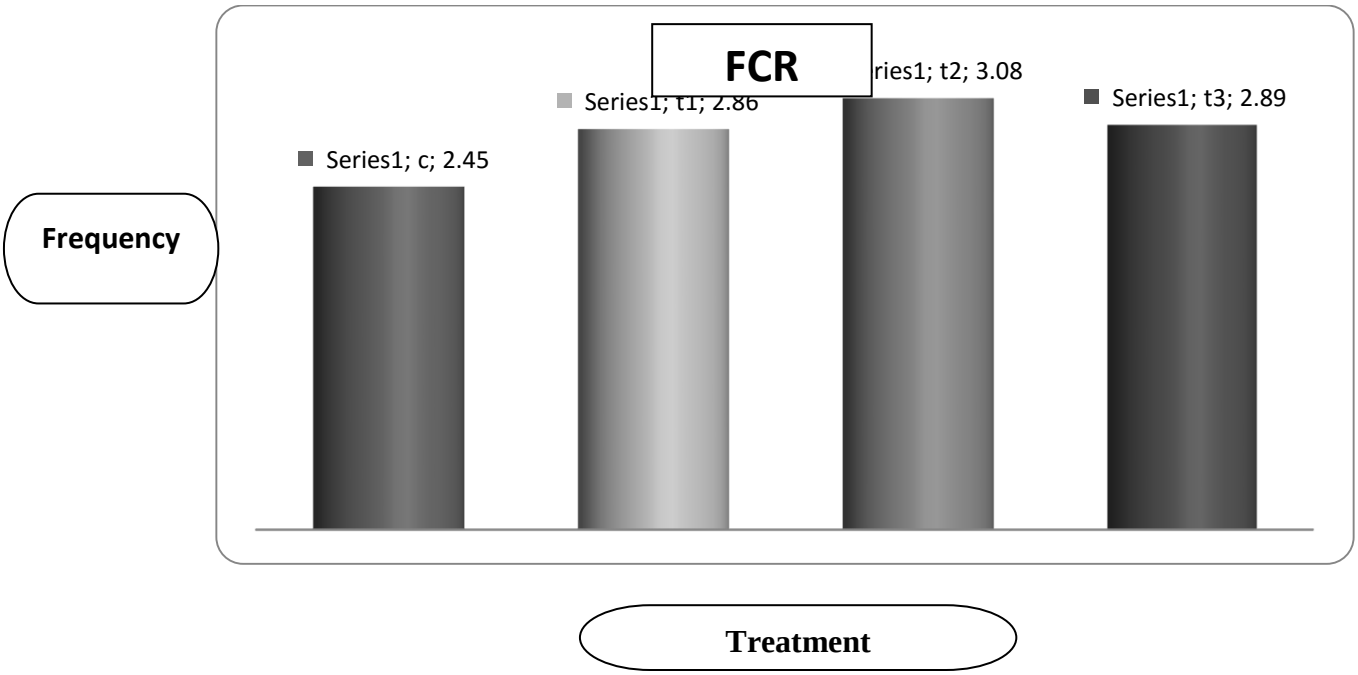

Figure (4.1) FCR One of Growth performance for O.niloticus fed different level of fish silage

CHAPTER FIVE

DISCUSSION

Results show in Table 4.1 indicated that final body weight (BW), weight gain (WG) and specific growth rate (SGR) of *O. niloticus* increased with increasing level of fish silage in diets. WG was found 7.90, 7.01 and 7.98 for T1, T2 and T3 respectively.

T0 Control shown (WG 5.99 SGR 1.72 WG% 11.42 and FCR 2.54 **Water quality parameters: pH** 6.84 **D.O** 5.55 **Temperature** 29.48 **NO_2** 0.00 **NH_3/NH_4** 0.00 **PO_4** 0.00 **NO_3** 0.00)

T1 shown second higher WG compared to treatments included control this results WG7.90 SGR1.98 WG% 13.39and FCR 2.89 **Water quality parameters: pH** 6.67 **D.O** 5.09**Temperature** 29.76 **NO_2** 0.00 **NH_3/NH_4** 0.00 **PO_4** 0.00 **NO_3** 0.00)

T2 shown lesser WG compared to treatments included control this results WG7.90 SGR1.98 WG% 13.39and FCR 2.89 **Water quality parameters: pH** 6.72**D.O** 4.06**Temperature** 29.44**NO_2** 0.25 **NH_3/NH_4** 0.25 **PO_4** 0.00 **NO_3** 0.00)

T3 shown higher WG compared to other treatments included control this results disagreement with Fagbenro and Jauncey (1998) studied the nutritional value of diets containing microbial fish silage partially dehydrated by the addition of soy meal, poultry by-products, or bone and meat powder, and found no significant differences in the performance and protein use when compared to diets based on fish meal. The experiment showed that these diets, especially the ones including silage and soy meal, could be used to feed tilapias, *Oreochromis niloticus* (omnivorous), and the North African catfish, *Clarias gariepinus* (carnivorous), with no changes in its performance, use of Proximate analysis of *O. niloticus* for *O.niloticus* fed different level of fish silage significantly ($P<0.05$)

increased crude protein when compared to the control group, while ash content and fat content decreased. In salmon farming, the search for diets that promote fast growth, favour fish sanitation, result in quality products and have low cost is essential. Some authors, trying to evaluate the use of fish silage in salmon (*Salmo salar*) feeding, concluded that although this silage did not promote a better development, it did not cause a decrease either, and its cost was much lower (**Espe et al.; 1994; Heras *et al.;* 1994).**

Supplementation of fish silage with different level to studied fish diets affect specific growth rate (SGR)., similar to results of fish silage use in the feeding of fish has been widely studied. Due to the similarity of this protein source with the raw material and low cost, especially when compared to fish meal, silage has a high potential use in aquaculture. (Goddard and Perret, 2005).

Water quality parameters: PH 6.68 D.O 5.42 **Temperature** 29.67 **NO_2** 0.25 **NH_3/NH_4 0.50 PO_4 0.00 NO_3 0.00**.

Chapter six

Conclusion

6.1 Conclusions:

Fish fed different level of fish silage exhibited numerically good growth performance and better feed utilization efficiency. Moreover, diets with fish silage were comparable to the positive control diet, as these were found to be efficiently utilized by *O. niloticus* fingerlings as well. Therefore, fish silage may pose as a potential candidate ingredient for fishmeal replacement in *O. niloticus* feeds.

Chapter seven

Recommendations

6.2 Recommendations

For further study, it is recommended that

- Conduction more experiment on silage diets to confirm this result.
- Use silage method utilize remnants of fish
- Use fish silage included as a substitute fish meal

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Plate:

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Plate of plastic aquariums

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Plate of water quality kids