Sudan University of Science and Technology College of Animal Production Science and Technology Department of Fisheries and Wildlife Science

EFFECT OF PUMPKIN SEED MEAL ON GROWTH PEFORMANCE, FEED UTILIZATION AND PROXIMATE

COMPOSITION OF *O.niloticus* **FINGERLINGS أثز إضافة وإستخدام بذور القزع في هعدالت النوو ألصبعيات البلطي النيلي**

A Thesis submitted in partial Fulfillment of the Requirement

Of the B.sc.Degree in Fisheries and Wildlife Science(Honor) By:

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Quran

بسم الله الرحمن الرحيم قال تعالى: "وهو الذي سخر البحر لتأكلو منه لحما طريا وتستخرجوا منه حلية تلبسونها وتري الفلك مواخرا فيه ولتبتغوا من فضله ولعلكم تشكرون" صدق الله العظيم

سورة النحل الاية (14)

ABSTRACT

The Present study was designed to evaluate the effect of different dietary Pumpkin seed meal levels on water quality ,growth performance ,feedUtilization and proximate composition of o.niloticus .TheStudy was performed in 12 experimental treatments plastic aquarium atdensity of 20 fish in groups aeration was provided by an air pump for each pond .The experimental nitrogenous (control 23.03% , T1 16.89%T2 20.04% , T3 16.19%). Diet were formulated the control diet had No pumpkin seed meal dite 1-3 were formulated to be $T1(10\%)$, $T2(20\%)T3(40\%)$ pumpkin seed meal. Fish was feed at level of 9% of body weight 3 time day at (9,13and 17 o clock)for 11 week .Data were statistically analyzed using ANOVA one-way analysis of variance comparisons among means were made by (LSD)when significant F-values were observed $(p<0.05)$, using SPSS version(21). Results obtained are summarized in the following :tilapia fed with diets contain 10% pumpkin seed meal had significant lower $(P<0.05)$ final weight Tilapia fed with diet contains 40% pumpkin seed meal had the highest final weight .The values of feed conversion ratio (FCR) showed significant Difference (P>0.05) between the dietary treatment fed to the fish.

Key words: Pumpkin seed, Water quality, Growth, body weight ,Tilapia.

المستخلص

تهدف الدراسة الى تركيب وتقيم أثر إضافة مسحوق بذور القرع بمستويات مختلفة عل جودة المياه ومعدلات النمو واستخدامه في التغذية والتركيب التحليلي لاسماك البلطي النيلي.

صممت التججربة في 12 أحواض بلاستيكية بكثافة 20 سمكة لكل حوض ومزود بتهوية.

التجربة (الكنترول 23.02% ، 16.89 ، 16.19% ، 16.19%.

جهزت العليقة الكنترول من غير إضافة بذور القرع أما D1 بإضافة نسبة 10% و D2 بنسبة 20% و 43 40% من مسحوق بذور القرع.

تم تغذية الاسماك بنسبة 9% من وزن الجسم على ثلاث مرات في اليوم (17.13.9) ساعة لمدة 30 يوم.

النتيجة اظهرت متوسط انحراف معياري وتم التحليل الإحصائي للبيانات باستخدام برنامج ANOVA وللمقارنة بين المتوسطات المستخدمة برنامج (LSD) وتم ملاحظة اٌمُ١)0.05>P)بإسخخذاَ بزٔاِج SPSS.

اظهرت الدراسة الآتي:

الْبِلْطي الّتي تم تغذيته بعِلْفة تحتوي على نسبة 10% من مسحوق بذور القرع ادى الي انخفاض كبير (0.05>P) للوزن النهائي، البلطي المغذي على عليقة تحتوي على نسبة 40% أعطت اعلى وزن نهائي.

قيم التحليل الغذائي اظهرت اختلافات معنويه (P>0.05) بين العلائق المعالجة المغذية للأسماك

كلمات مفتاحية :بذور القرع،جودة الماء،نمو،وزن الجسم ،بلطي .

Dedication

To our parents for their encouragement. To our brother and sister and friends To all whom we love…

Acknowledgment

Our greatest thanks to Allah, the most Merciful who gave us the health, strength and patience to conduct this study . Grateful thanks to our supervisor Dr.Sara Boshara AL-Magboul and Ustaz . Fouzi Ali Mohmmed Department of Fisheries and Wildlife Science, College of Animal Production Science and Technology, Sudan University of Science and Technology for his guidance and provision of Scientific Knowledge .Finally our thanks to all there who helped us.

Table of content

List of Tables

List of figures

CHAPTER ONE

INTRODUCTION

The aquaculture sector is vital to maintain fish supply, especially with the decline in capture fish industry due to unsustainable practices **(FAO 2006).** However, aquaculture, which is highly dependent on fishmeal as a protein source for fish feeds, also proves to be no longer sustainable (**Tacon & Metian 2008**).

The steady supply of fishmeal and fish oil vis-à-vis the rapid expansion of aquaculture has contributed to a rise in costs of production, and in effect a rise in the cost of fish. This might be one of the factors associated with the continuous rise in the retail price of the low priced fish *O. niloticus* (Nile tilapia), one of the most dominant, farmed aquaculture species in the Philippines, and is also the subject of this research(**BAS2013**).

The rise of aquaculture production is thus highly important to fill in the gap between supply and demand, so as to keep the price of food fish at a reasonable range for the rural and urban poor which is critical for food security (**Huntington & Hasan 2009**).

In this context, the formulation of fish feed using a cheap, locally available and highly nutritious terrestrial resource such as Cucurbit maxima seeds is needed. Crude protein levels of C. maxima seeds and kernels are comparable to high protein-containing seeds and legumes such as soybeans and cowpea; while lipid content of C. maximakernels is comparable to sunflower, soybeans and cotton seeds (**Srbinoska et al 2012**).

Seed oils are mainly composed of polyunsaturated fatty acids, namely oleic and linoleic acids with the latter accounting for more than half of the seed's fatty acid content and its seeds also contain vitamins A, C and E calcium, iron, phosphorous and zinc, and cucurbitacins (**Raganathan et al2013**).

Pumpkin seed are widely grown in the southern regions of Austria (Styria province) and the adjacent regions in Slovenia and Hungary **(Idouraine et al., 1996)**.

The pumpkin seed is valued in regard to nutritional points. Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties **(Stevenson et al., 2007**). The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids **(Stevenson et al., 2007).** The pumpkin seed is a good source of potassium, phosphorus and magnesium, and also contains moderately high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc, and copper) and these elements make pumpkin seed valuable for food supplements **(Lazos, 1986).**

Objectives:

- 1. Evaluate the effect of different dietary pumpkin seed meal leve on water quality .
- 2. Growth performance .
- 3. Feed utilization and proximate composition of *O.niloticus* fingerlings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture

According to the 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 tons correspond to aquaculture production.

Sixty percent of the fish captured worldwide are used in the fresh fish market or processed as frozen, canned or cured foods, causing a considerable amount of waste material. The volume of waste produced by processing plants is calculated to be approximately 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 for being refused by Federal/State Inspections in fresh commercialization markets **(Rebeca et al, 1991**).

The growth of aquaculture associated to improvements in increasingly intensive cultivation practices has intensified the demand for high quality foods **(Espe and lied 1999)** which allow a formulation of highly nutritive, economically viable and ecologically correct diets.

As in any zoo technical culture, feeding is accountable for a high percentage of the operational cost in fish farming, reaching indices of 40 to 60%. Protein ingredients contribute to the largest part of this cost (**Cheng et al, 2003**).

3

The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems and use of quality feeds, which meet the nutritional requirements of cultured fish (**FAO, 2006**). Stimulated by higher global demand for fish, world fisheries and aquaculture production reached 157million tons in 2012 and is projected to reach about 172 million tons in 2021, with most of the growth coming from aquaculture (**FAO, 2013).** This increase of aquaculture production must be supported by a corresponding increase in the production of designed diets for the cultured aquatic animals (**Rahman et al., 2013**).

2.2 Nile tilapia

Tilapia is the common name given to three genera of fish in the family Cichlidae namely *Oreochromis*, *Sarotherodon* and Tilapia (Santiago and Laron, 2002). The genus *Oreochromis* includes Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia, (*Oreochromis mossambicus*) and blue tilapia (*Oreochromis aureus*). Regionally, tilapia is the most preferred cultured fish in East Africa but are the second most important cultured fish in the world after carps (Dan and Little, 2000; El-**Sayed, 2006**). The culture of tilapia started as early as 2000 – 2500 BC (Chimits, 1957). Since then the growth trend of cultured O. niloticus has increased consistently (Figure 1). Today, more than 22 tilapia species are being cultured in many tropical and subtropical regions with an expanded penetration of a variety of tilapia products in markets (**El-sayed, 2002**). Indeed, there are not any reports of cultural or religious restrictions on tilapia consumption all over the world (**Fitzsimmons, 2000**).

Tilapia growth is attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on wide range of foods.

Nile tilapias are relatively inexpensive fish to feed unlike other carnivorous finfish thanks to their low trophic feeding level. In addition, tilapia are similar to channel catfish (*Ictalutus punctatus*), in that they can tolerate higher dietary fibre and carbohydrate concentrations than most other cultured fish (**El-Sayed and Teshima, 1992**). However, to ensure high yield and fast growth at least cost, a well balanced prepared feed is essential to successful tilapia culture. Even though slight variations exist among tilapia species, nutrient requirements are primarily affected by the size of the fish (**El-Sayed and Teshima, 1992**).

2.3 Tilapia fishmeal alternatives

The development of sustainable aquaculture depends on the establishment of alternative feedstuffs to FM (**Olukayode and Emmanuel, 2012**). Since the success of fish farming depends on the provision of suitable and economical fish feeds, we need to use locally available feedstuff especially agricultural by-products to reduce the price of complete feeds (**Fagbenro, 1999**). Due to the rising cost of commercial tilapia feeds, farmers are looking for alternative feeds in order to make aquaculture a viable and attractive venture (**Hossain et al., 2002**). What therefore are the possible solutions to the FM dilemma in tilapia feed? Research has not only shown several alternative protein sources for FM in tilapia feeds but has also identified the essential nutrients in FM and a way of incorporating them into the alternatives (**Tacon et al., 1983**). During the last 3 decades, studies have revealed that warm water fishes require both n‐6 and n‐3 fatty acids while cold‐water fishes require only n‐3 fatty acids for optimum growth and development (**Takeuchi, 2008**). Whereas optimal use of the fish resources (trash fish) and exploiting underutilized ocean resources such as Antarctic

krill have been proposed, the most significant option could be the use of terrestrial animal meals and plant protein-rich derivatives (Tacon and Metian, 2008). Experts have further proposed other protein source such as single cell proteins, earthworm, insects, snails, maggots, and frogs as potential fish meal replacers (**Tacon et al., 1983).** However, their sustainable production and effective use in aquaculture need further economic scrutiny. Even though **El-sayed and Tacon (1997)** explained how animal products, plant protein derivatives and single cell proteins can be possible FM replacers in tilapia feeds, it is important to know whether these alternatives can completely replace FM without compromising production. This subject has been discussed by aquaculture nutritionists, fish biologists and fish farmers albeit with limited consensus. According to **Jackson's (2009)** Fish in-Fish out (FIFO) ratio concept, which technically considers how much forage (catch) fish needed to produce 1kg of cultured fed fish species, it would require only 0.3 kg of FM to produce 1kg of tilapia. This implies that complete FM replacement could be a scientific reality. Below we review the possibilities of complete FM replacers in Nile tilapia grow-out diets. The specific focus on their nutritive values, availability and economic feasibility has been also discussed.

2.3.1 Animal meals

The main terrestrial by-product meals, which have been tested as FM replacers for tilapia include poultry by-product meal (PBM), feather meal (FeM), blood meal (BM), and meat and bone meal (MBM). Despite their usually high crude protein content, they are usually deficient in one or more essential amino acids (EAA) whereby the limiting EAAs generally being lysine, methionine and isoleucine (**Tacon and Jackson 1985**). However,

these imbalances can be overcome by mixing complementary protein byproduct meals so as to obtain the desired EAA profile (**Davies et al., 1989**). The animal proteins are free of anti-nutritional factors, are palatable, cheaper and readily available than fishmeal thus making them perfect FM replacers for tilapia especially in developing nations (**El-Sayed, 1999).**

2.3.2 Blood Meal (BM)

BM is an animal waste product readily available in abattoirs and can be used as an alternative high quality and cheap protein source in fish feed formulation. BM products can effectively replace marine proteins in growout rations for shrimps (*Pannaeus vannamei*) when supplemented with methionine (**Dominy and Ako, 1988**). **Davies et al. (1989)** fed Mozambique tilapia (*O. mossambicus*) fry for seven weeks using BM and found that up to 75% of the FM in the diets could be effectively replaced. However,(**Otubisin 1987**) conducted a 120 day experiment with caged O. niloticus fingerlings using BM and concluded that dietary BM inclusion levels above 50% of the FM protein significantly reduced fish performance. **El-Sayed (1998)** also found that BM used as a sole protein source in practical diets for Nile tilapia reared in outdoor concrete tanks for 150 days resulted to reduction in fish performance. It is important to realise that management factors such as feeding frequency, rearing condition and other environmental factors are equally important. **(Agbebi et al. 2009)** reported that fish meal can be replaced completely by BM with no adverse effect on growth, survival and feed conversion of Nile tilapia and *Clarias gariepinus* juveniles.**(Hussain et al. 2011)** found that nutrient digestibility values of FM and BM for dry matter and crude fat are significantly similar for most tilapia fish species hence BM can be a perfect FM replacer in the diets. The

studies of (**Aladetohun and Sogbesan 2013)** showed that inclusion of BM in the experimental diet improved the growth performance of Nile tilapia (Table 1). The summary of growth performance parameters of the experimental feeding using different BM inclusion levels are shown in Table 1 and Figure 5. Despite the difficulty in quantifying the amount of BM produced in Kenya or larger East Africa, BM is cheap, readily available and allowed by most governments of developing countries. The authors suggest that this could be the forgotten asset that can be used to formulate least cost fish feeds to lower production expenses and maximise profit in aquaculture. However, more studies are recommended for the BM feed formulation procedure to address issues of possible disease transfer from livestock to fish and human. Indeed, this is a lucrative opportunity for research collaborations in Kenyan aquaculture industry.

2.3.3 Meat and Bone Meal (MBM)

MBM is a by-product of the animal rendering industry, which is less expensive than FM but more expensive than BM. According to **(Kellems et al. 1998)**, MBM is an excellent source of supplemental protein, calcium, vitamin B-12 and phosphorus with a well-balanced amino acid profile and high protein digestibility to most fish .However, MBM is somewhat lower in some amino acids content and higher in minerals as compared with FM and BM (**Yang et al., 2004**). Nevertheless, the frequently reported variations on composition of these protean meals could be largely due to variability in raw material composition and quality. MBM has been used effectively in feeds for a variety of fish species, such as rainbow trout (**Bureau et al., 2000**), red drum (**Kureshy et al., 2000**), Australian snapper (**Quartararo et al., 1998**) and Nile tilapia (**Fasakin et al., 2005**). Even though the ash content may

limit the use of MBM in fish feeds (**Fasakin et al., 2005**), research has demonstrated that MBM can partially or totally replace FM when used at levels of 5% to 15% in fish feeds (**Kellems et al., 1998**). **(Tacon et al. 1983)** found that MBM supplemented with Methionine successfully replaced up to 50% FM protein within diets containing 45 % crude protein fed to *O. niloticus* fry over a six-week period. (**El Sayed and Tacon 1997)** found that MBM could effectively replace up to 75% of the FM in diets fed to *O. mossambicus* fry over a seven-week period. In fact,(**El Sayed and Tacon 1997)** reported that diets containing MBM or high MBM/BM ratios were superior to FM even at a 100% substitution level. Even though MBM is reported to be about 10% lower in protein and energy digestibility than FM (Table 2), this has insignificant impact on tilapia growth **(Hanley, 1987)** hence MBM could be a perfect complete FM replacer **(Yang et al., 2004).** However, due to fear of diseases transfer, some countries have restricted the feeding of MBM and some only allow MBM derived from monogastric animals to be fed only to ruminant animals and vice versa **(European Community, 2002).** In addition, the sustainability of MBM as fish feed ingredient could be threatened by the stiff competition from human. The use of MBM may need to be limited due to water quality considerations (**Hardy, 1996**).

3.3.4 Poultry By-product Meal (PBM)

PBM is made of ground, rendered, or clean parts of the carcass of slaughtered poultry. PBM has been tested at varying success so far in salmon (**Yang et al., 2004**), tilapia (**El-Sayed, 1998**), sea bream (Nengas et al., 1999), channel catfish (Sadiku and Jauncey, 1995a) and common carp (Hasan et al., 1993). PBM is similar to FM in composition except being slightly lower in some amino acids (Table 2) (Yang et al., 2004). FM and

PBM are generally highly digestible in protein (88%) and energy (82%) (Table 3). These digestibility values suggest that PBM could be used in aqua-feeds to a level similar to FM (Yang et al., 2004). Referring to proximate analysis (Table 2), the protein, fat, calcium and phosphorus contents in PBM are comparable to those of FM, suggesting possibility FM replacement by PBM. El-Sayed (1998) found that red tilapia and Nile tilapia can efficiently utilize PBM as single dietary protein sources. Belal et al. (1995) fed *O. niloticus* fingerlings with diets containing 0 – 20% chicken offal silage (COS) made from chicken viscera, as a replacement of FM. They found that the growth and body composition of fish fed COS were similar to that of fish fed with FM based diet. Similarly, Gaber (1996) found that the growth of Nile tilapia fingerlings fed with PBM as a protein source replacing FM up to 40% level was better than that of those fed 100% FM. It is therefore possible that PBM could totally replace FM in Nile tilapia diets.

Indeed, this is an interesting observation that should attract the attention of aquaculture nutritionists. However, more studies should be focused on the sustainable exploitation of PBM especially in developing countries. **Feather Meal (FeM)** Feather meal is a by-product from poultry production. FeM contains a complex protein (keratin), which can be hydrolysied to improve bio-availability (Munguti et al., 2014). FeM is an economical protein ingredient mostly used in aqua-feeds (Poppi et al., 2011). FeM is rich in amino acids such as cystine, threonine and arginine, and has high level of pepsin digestible protein (Fowler, 1990). The amino acid profile of FeM is similar to those of FM and soybean meal **(Fowler, 1990)**. FeM has been used to feed many fish species including shrimps **(Fowler, 1990; Steffens, 1994; Bureau, 2000)**, salmon and African catfish **(Fowler, 1990).** However, the utilization of FeM in fish diets is limited due to a complex protein called keration **(Steffens, 1994)**. The utilization of hydrolyzed FeM protein in tilapia feeds could be economically feasible. However, studies conducted to evaluate the use of hydrolysed FeM in fish diets recommend low substitution levels due to poor digestibility and suboptimal levels of essential amino acids **(Steffens, 1994; Mendoza et al., 2001).** Due to this, studies show that combination of hydrolysed FeM and papaya leaf meal (PLM) may promote the feeding value of FeM and by extension promote its use in Nile tilapia feeds and that complete replacement of FM with hydrolysed FeM dietary levels and PLM cannot significantly affect growth of Nile tilapia reared in cages **(Munguti et al., 2014).** (**Arunlertaree and (Moolthongnoi 2008)** concluded that fermented FeM could be used at 25 % up to 50 % as the replacement of FM for 30 % CP Nile tilapia diet.

2.3.5 Plant protein-rich derivatives

Plant proteins are almost similar to FM in terms of the protein content and protein and amino acid digestibility **(Hardy, 1996).** However, their amino acid profile does not match the amino acid requirement of some fish species as FM does (see figure 6) **(Hardy, 1996).** For example Methionine is the limiting amino acid in soybean meal (SBM), while corn gluten meal is deficient in lysine **(Gallagher**, **1994)**. Wheat gluten meal is limited in lysine and arginine **(Gallagher**, **1994)**. So far, nutrition research has concentrated on the replacement of animal protein by plant proteins **(Liti et al., 2006)** but the palatability of many plant materials is hindered by presence of antinutritional factors and low bioavailability **(Francis et al., 2001).** Some plant proteins contain phosphorus phytate, which binds phosphorus, reduces palatability and interferes with the bioavailability of divalent trace elements

(Gallagher, 1994). Nevertheless plant based feeds containing soybean meal protein, canola meal, extruded pea seed meal, wheat and corn meal supplemented with lysine and methionine has been used in the formulation feeds for catfish, tilapia and carps without affecting their growth performance **(Tacon and Metian, 2008).**

2.3.6 Soy Bean Meal (SBM)

So far, SBM is the best plant protein source in terms of protein content (Table 4) and EAA profile (Figure 6). Scientists have considered SBM as a partial or total FM alternative for tilapia, with varying results. SBM could replace between 67 and 100% of FM, depending on fish species, dietary protein level, source, processing methods and culture system used **(El Sayed, 1999).** With or without Methionine supplementation, SBM successfully replaced up to 75% of FM in test diets fed to Nile tilapia fry **(Shiau et al., 1989)** suggesting that supplementing SBM with the limiting EAA could be insignificant. Studies of **(Viola and Zohar 1984)** also reported that supplementing tilapia diets with crystalline EAA did not improve Nile tilapia performance. This implies that minerals, rather than limiting EAA, may be the limiting factors in the efficient utilization of SBM for tilapia **(El Sayed, 1999). (Viola et al. 1988)** found that the growth of tilapia hybrids (*O. niloticus x O. aureus*) fed 100% SBM diet supplemented with Lysine, Methionine, oil and di-calcium phosphate was similar to that of fish fed 100% FM diet. Also, the non-inclusion of the limiting EAA to SBM diet did not affect growth and SBM supplemented with 3% di-calcium phosphate and oil completely replaced FM without any adverse effects on tilapia growth **(Viola et al., 1988). (Davis and Stickney 1978)** found that the inclusion of SBM at 15% dietary protein level impaired growth of blue

tilapia, while at 36% protein; SBM could totally replace FM in the diets without significant growth retardation. The contradiction among researchers regarding the use of SBM as a protein source for fish may be related to the quality and processing of SBM, fish species, size and culture systems. Even though SBM contain some anti-nutritional factors (trypsin) **(El Sayed, 1999),** thermal processing can be used to make quality feeds out of SBM **(Tacon, 1993). (Wassef et al. 1988)** found that the germination and defattening of SBM reduces the activity of protease inhibitors. Similarly, heating SBM destroys the anti-nutritional factors and improves nutrient bioavailability **(Tacon and Jackson, 1985).** The authors recommend blending SBM with grain protein concentrates to adjust the amino acid profile to overcome limitations of individual plant proteins. **(Sadiku and Jauncey 1995)** recommended mixing SBM with animal protein sources to improve its quality for Nile tilapia. The safety and use of genetically modified soybean meal (GM SBM) as fish feed protein source has been demonstrated **(Suharman et al., 2009).** Studies have shown that there are no differences in fish growth, survival, feed conversion, and fillet composition between the fish fed GM soybean and non GM SBM for Nile tilapia **(Suharman et al., 2009).** According to **(Watanabe 2002),** defatted soybean meal is universally accepted, both qualitatively and quantitatively, has favourable amino acid profile compared with other plant protein sources. Soybean meal is consistently available, cost-effective, and reported to be palatable to most fish species **(Watanabe, 2002).** Based on these benefits, several aquaculture nutritionists should aim at totally replacement of FM with SBM as a protein source because many results have indicated that SBM is one of the most promising FM replacements **(Watanabe, 2002).**

2.3.7 Cotton Seed Meal (CSM)

CSM contains good protein contents and amino acid profile (Table 4) depending on processing methods (El sayed, 1999). However, CSM is limited in Cystein, Lysine and Methionine and has high content of gossypol, an anti-nutrient compound, which may limit the use of CSM in animal feeds (El Sayed, 1999). The use of CSM as protein sources for tilapia has registered mixed results. **(El-Sayed 1990)** successfully used prepressed solvent extracted CSM as a single dietary protein source for Nile tilapia and they performed better than FM fed fish. However, **(El-Sayed 1990)** found that O. niloticus and O. aureus fed on CSM-based diets grew at slower rates compared to fish fed FM-based diets, probably due to the gossypol and cyclopropionic acids contained in CSM. The studies of **(Viola and Zohar 1984)** reported that about 50% CSM successfully replaced SBM in diets fed to tilapia hybrids reared in floating cages. **(El-Sayed 1987)** found that Tilapia zillii grew best on diets containing 80% CSM protein. **(El-Sayed and Kawanna 2008)** found that CSC (42% CP) used as the only feed input for Nile tilapia reared in earthen ponds, fertilized with cattle manure for 100 days, resulted in a sharp increase in fish weight. In addition to the use of CSC as a protein source in commercial pelleted feeds for tilapia, it can be used as a source of fertilizer in semi-intensive tilapia culture to increase natural food production within fish ponds **(El Sayed, 1999)**. CSM is readily available and cheap plant protein source in most parts of the world.

2.3.8 Other oilseed plant by-products

There are many oilseed by-products such as groundnut, sunflower, rapeseeds, sesame seeds, macadamia and palm kernel, which can be utilized as alternative protein sources for tilapia. Despite their good protein contents

and EAA profiles (Figure 6), not much literature is available on their use as complete FM replacers. **(Jackson et al. 1982)** found that 25, 75, 75 and 50% of groundnut cake, sunflower meal, rapeseed meal and copra meal respectively could replace FM protein without significant effect on *O. mossambicus* growth. However, **(Davies et al. 1989)** reported that only 15% rapeseed meal could effectively replace FM in *O. mossambicus* diets, while higher levels resulted in poor growth due to the high content of glucosinolate (anti-nutrient) in rapeseed. When Nile tilapia fingerlings were fed up to 60% palm kernel meal, the performance was similar to those fed 100% FM diet **(Omoregie and Ogbemudia, 1993).** Macadamia press cake was successfully used as a protein source for Nile tilapia by **(Fagbenro 1999)** who found that the growth of tilapia fed 33.4% CP Macadamia cake in concrete tanks for 180 days was similar to those offered a commercial 35.5% CP FM diet. The low price of MC favours it as a promising alternative plant protein source for tilapia.

2.4 Aquatic plants

Several species of aquatic plants could be potential sources of FM replacers in aquaculture. However, studies conducted on the use of aquatic plants in tilapia feeds have produced varying, and sometimes, conflicting results. When fish feed made of *Azolla pinnata* was used as a FM replacer for Nile tilapia fingerlings and adults respectively, at $0 -100\%$ substitution levels, fish fed with *Azolla pinnata* showed extremely poor performance even at the lowest inclusion level of 25% **(El-Sayed et al., 2000).** Similar results were reported for *T. rendalli* fed *Azola* microphylla **(Micha et al. 1988).** However**,(Naegel 1997)** found that up to 30% of FM diet fed could be successfully replaced with dried *Azolla* meal for Nile tilapia. Moreover,

(Santiago et al. 1988a) reported that a diet containing up to 42% of Azola pinnata produced better growth rates of Nile tilapia fry than did the control FM diet. Fresh duckweed (family: Lemnaceae) is a good food source for tilapia, as it contains about $35 - 45\%$ CP with good AA and mineral profiles **(Mbagwu et al., 1990).** Production of Nile tilapia using duckweed (Lemna and Wolffia) feed as a single nutritional input in earthen ponds was very successful in Bangladesh, netting up to 7.5 metric ton ha-1 year-1 **(Skillicorn et al., 1993)**. The EAA index value of 81 for raw Spirulina is scientific evidence that it is an adequately nutritious food source for larval tilapia **(Takeuchi et al., 2002).** It can be concluded that the availability of adequate supply of Spirulina during the early stages is important for the normal growth and development of larval tilapia **(Takeuchi et al., 2002).** Nevertheless, complete replacement of FM using aquatic plants needs further scientific investigations including in and outdoor trials.

2.3.5 Single Cell Proteins (SCP)

In the recent past, biosynthesis and utilization of SCP, which are a group of microorganisms including unicellular algae, fungi, bacteria, cyanobacteria and yeast by tilapia within culture systems has attracted the attention of aquaculture nutritionists **(El-Sayed, 1999; Avnimelech, 2007)**. The concept of heterotrophic food web indicates that fish can be fed directly or indirectly on primary producers and also have a chance to feed on bacteria degrading residues present in the pond **(Avnimelech, 2007)**. The main principle of SCP is to recycle nutrient by maintaining a high carbon / nitrogen (C: N) ratio in the water in order to stimulate heterotrophic bacterial growth that converts ammonia into microbial proteins, also known as biofloc technology (**Avnimelech et al., 1989; Azim and Little, 2008).** As a byproduct, bacteria produce between 60-600 kg ha-1 day-1 of protein for fish **(Avnimelech, 1999).** Indeed authors agree that SCP produced using cheap carbon and nitrogen sources can partially or completely replace expensive commercial protein sources in *O. niloticus* feeds **(Dempster et al., 1995).** SCP contains more than 38% protein, 3% lipid, 6% fibre, 12% ash and 19 K J g-1 energy, which is just sufficient for tilapia production **(Azim and Little, 2008).** Proximate analysis of biofloc sample collected from tilapia SCP fed system revealed promising nutrient contents (Figure 7) **(Widanarni et al., 2012).** The biofloc contains up to 10–25% lipid content, which is the optimum dietary lipid requirement for tilapia **(Widanarni et al., 2012)**. Indeed these values are far much better than most commercial pellet feeds used in aquaculture farms today **(Ogello et al., 2014).** Fish fed with 20% CP of SCP based diet significantly performed better than those fed commercial 30% FM diet (Table 5). The active recirculation of proteins by microorganisms is credited for the increased protein utilization in fish reared in SCP fed systems **(Ogello et al., 2014).** This is definitely positive information to farm managers who may even aim at increasing further recycling of proteins. However, more studies are needed to ascertain the sustainability and the nature of bacteria contained in the SCP production systems. Several authors have performed studies on many other potential FM replacers for Nile tilapia production (see table 6).

2.3.6 **Economic feasibility of FM alternatives**

Evaluation of FM replacers in tilapia feeds has mainly taken biological and nutritional points of view with limited economic studies. Despite mixed results obtained from the FM replacers, cost benefit analyses indicated that they are economically better. For example, economic

evaluation of cotton seed meal **(El-Sayed, 1990),** corn gluten feed **(Wu et al., 1995)** and animal by-product meal **(El Sayed, 1998)** as protein sources for Nile tilapia indicated that cost and profit indices of these protein sources were better than for FM-based diets. An analysis of the cost implication of replacing FM with BM revealed that 100% BM inclusion was significantly cheaper compared to 100% FM diet **(Aladetohun and Sogbesan, 2013).** It cost 0.62 USD to produce 64g of tilapia within 3 months using 100% BM while 0.79 USD was spent to produce 30g of tilapia using 100% FM diet over the same period **(Aladetohun and Sogbesan, 2013).**

2.3.8 Pumpkin seed

Vegetable oils are essential in meeting global nutritional demands and are utilized for many food and other industrial purposes **(Idouraine** *et al.* **1996).** Despite the broad range of sources for vegetable oils, the world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively **(Stevenson** *et al.,* **2007).** These conventional sources of vegetable oil no longer meet the ever increasing demands of domestic and industrial sectors **(Idouraine** *et al.,* **1996).**

Therefore, the need exists to look for other sources to supplement the supplies. From this view point, non-conventional oilseeds are of much concern to cope this challenge.More recently, research activities have focused on examining and characterizing new sources of edible oils. **(Esuoso** *et al.***1998)** reported that seeds of some species of Cucurbitaceae can be the edible oil sources to meet the increasing demands for vegetable oil.

Pumpkins belong to the family Cucurbitaceae. The majority of the species

in this family are used as food and are found in five genera: *Citrullus* (water melons and wild colocynths), *Cucumis* (cucumbers,gherkins and melons), *Lagenaria* (gourds),*Sechium* (chayotte) and *Cucurbita*. The genus *Cucurbita,* which is economically the most important one, includes five species: *C.maxima, C. pepo, C. moschata, C. ficifolia,*and *C. turbaniformis* in which *C. pepo*exhibits the widest variation, especially with respect to fruit characteristics (Gemrot *et al.,2006*). *C. pepo* is a native species of North

America and has been cultivated there for several thousand years **(Paris, 1989).** It is claimed that *C. pepo* is more persistent and less liable to deterioration, which certainly is reflected in the quality of the extracted oil **(Markovic and Bastic, 1975).** Hull-less or naked pumpkin seed are widely grows in the southern regions of Austria (Styria province) and the adjacent regions in Slovenia and Hungary **(Idouraine** *et al.,* **1996).** The pumpkin seed is valued in regard to nutritional points. Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties **(Lazos 1986; Stevenson** *et al.,* **2007).** The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids **(Stevenson** *et al.,* **2007)**. The pumpkin seed is a good source of potassium, phosphorus and magnesium, and also contains moderately high amounts of other trace minerals **(calcium, sodium, manganese, iron, zinc, and copper)** and these elements make pumpkin seed valuable for food supplements **(Lazos, 1986).**

Raw or roasted pumpkin seeds are used as a snack food for human consumption in many cultures all over the world. The kernels of pumpkin seeds have been utilized as flavor enhancers in gravies and soups, and used

in cooking, baking and ground meat formulations as a nutrient supplement and a functional agent **(Tsaknis** *et al.,* **1997;El-Adawy and Taha, 2001).** The oil of pumpkin seeds are being used as a cooking oil in some countries in Africa and the Middle East, and as a salad oil in the south of Austria and the adjacent regions in Slovenia and Hungary **(Wenzl** *et al.,* **2002).**

The pumpkin seeds possess valuable dietary and medicinal qualities besides being the source of good-quality edible oils. Pumpkin seed oil has been used traditionally as medicine in many countries such as China,Yugoslavia, Argentina, India, Mexico,Brazil, and America. It is applied in therapy of small disorders of the prostate gland and urinary bladder caused by hyperplasia (BHP). Pumpkin seed extract has been reported to have antidiabetic, antitumor, antibacterial, anticancer, antimutagenic, and antioxidant activities. It has also been found to have strong hypotriglyceridemic and serum cholesterol-lowering effects **(Fu** *et al.,***2006).** The health benefits of pumpkin seeds are attributed to their macroand microconstituent compositions. They are a rich natural source of proteins, triterpenes,lignans, phytosterols, polyunsaturated fatty acids, antioxidative phenolic compounds, carotenoids, tocopherol, and minerals **(Fu** *etal.,* **2006).**

CHAPTER THREE MATERIAL AND METHODS

3.1 Study area

The present study was carried out in fish hatchery at Department of Fisheries science and Wildlife, College of Animal Production Science and Technology, Sudan University of Science and Technology.

3.2 Material :

1. sieve 2.basin 3.big plate 4.chopper 5.sensitive balance 6.mill 7.small net 8.crucible 9.air pump

3.3 Experimental design and conditions

240 Nile tilapia fingerlings were distributed in 12 experimental treatments in plastic aquarium at a density of 20 fish in groups. Aeration was provided by an air pump for each pond. Water was changed partially every 3 days and entirely every week. Fish was fed at a level of 9% of body weight three times a day (9, 13 and 17 o'clock) for 11week .

3.4 Experimental diets

Four experimental is nitrogenous (control 23.02 %, D1 16.89%, D2 20.04% and D3 16.19% CP) diet were formulated (Table 3.1). The control diet had no Pumpkin seed meal. Diets 1-3 were formulated to be D1 (10%) D2 (20%), D3 (40%) pumpkin seed meal. The dry ingredients were mixed together and incorporated into the feed diet components as shown in Table 1 (Salinas *et al*., 2005). After a desirable dough quality was obtained, diets were passed through a mincer with a die (2 mm diameter) and the resulting spaghetti-like strings were dried until the moisture levels were at approximately 10%. The diets were then stored in a -15ºC freezer until being used.

Table (3.1): Formulation and composition of the experimental diets (dry matter basis).

	Experimental diets					
Ingredient $(\%)$	control	T1		T2	T3	
Fishmeal	35	35		35	35	
Pumpkin seed	$\overline{0}$	10	20		40	
meal						
Groundnut cake	19.5	19.5	19.5		19.5	
Sorghum meal	10	5	5		$\overline{0}$	
Wheat bran meal	15	10	5		Ω	
Vegetable oil	06	06	06		06	
Mineral mix	1.5	1.5	1.5		1.5	
Vitamin mix	03	03	03		03	
Bread flour	10	10	5		5	
Total	100	100	100		100	
		Experimental diets				
Chemical composition		control	T1	T2	T3	
Dry matter $(\%)$		90.92	95.10	80.01	95.05	
Crude protein $(\%)$		23.02	16.89	20.04	16.19	
Crude fat $(\%)$		5.05	5.05	10.01	10.13	
Ash $(\%)$		15.05	25.05	23.82	22.74	
Nitrogen free extract $(\%)$		37.76	33.06	26.18	20.89	

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:

3.3.1. 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.

3.3.2Dissolved 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 **П**

П.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 $= 0.65 \times$ [No. of drops of **D.O.5**]

3.3.3Total ammonia (NH3/NH4):

 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.

3.4.1 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 (%) = Initial weight – Dry weight \times 100 Initial weight

3.4.2 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:

Protein % = (Va – Vb) x N x 14 x 6.25 x 100 1000 x Wt

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

W_t = weight of sample

N= normality of NaoH

3.4.3 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:

Fat $\% =$ Extracted fat weight x 100 Sample weight

3.4.4 Ash Content Determination:

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

Ash $\% =$ Ash weight x 100 Sample weight

3.5 Determination 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) $\frac{\log x}{1 - x}$;

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

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

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

3.6 Statistical Analysis

Results were expressed as means \pm standard deviation (SD). Data were statistically analyzed using ANOVA one-way analysis of variance. Comparisons among means were made by (LSD) when significant F- values were observed $(P \le 0.05)$, using SPSS version (21).

CHAPTER FOUR

RESULTS

Parameters for growth performance and survival rate of Nile tilapia are presented in Table 4 and 2. Growth performance differ (P>0.05) between tilapia fed with the control diet and those fed will containing different level of pumpkin seed meal in terms of final weight, weight gain (WG), specific growth rate (SGR) and daily weight gain (DWG). However, tilapia fed with diets containing 10% pumpkin seed meal had significant lower (P<0.05) final weight, weight gain, specific growth rate and daily weight gain compared to the control diet. Tilapia fed with diet containing 40% pumpkin seed meal had the highest final weight. As for weight gain, specific growth rate and daily weight gain, significant difference were shown among tilapia fed the diets with different level of pumpkin seed meal and. The values of feed conversion ratio (FCR) showed significant difference (P>0.05) between the dietary treatment fed to the fish.

Parameters						
Treatments	D.M	C.P	Fat	Ash	N.F.E	
T1	12.51 ± 0.04 ^c	$10.59 \pm 0.127^{\circ}$ $23.09 \pm 0.014^{\circ}$ $6.68 \pm 0.014^{\circ}$ $47.13 \pm 0.169^{\circ}$				
T2		20.01 ± 0.014^a 14.615 ± 0.120^a 6.68 ± 0.007^d 7.00 ± 0.00^b 51.69 ± 0.084^a				
T3		17.68 ± 0.04^b 14.79 ± 0.13^a 11.78 ± 0.02^c 7.75 ± 0.07^b 48.01 ± 0.25^b				
Control		20.02 ± 0.02^a 10.94 ± 0.13^b 14.01 ± 0.014^b 10.10 ± 0.14^a 45.04 ± 0.16^c				
a,b,c,d: Means in the same column with superscript are significant different at						

Table (4.1): Proximate composition of O.niloticus feed different level of pumpkin seed meal.

Table :(4.2) Growth performance and feed utilization for O.nilotcus fed diets containing different levels of Pumpkin seed meal for 11 weeks. Level %

	Level %					
	0.0 (Control)	10	20	40		
Parameters	T ₀	T1	T2	T3		
Initial weight (g)	3.195 ± 0.178^b	4.498 ± 0.93 ^a	3.958 ± 0.177^b	3.643 ± 0.468^b		
Final weight (g)	7.917 ± 2.402 ^c	11.41 ± 2.786^b	14.98 ± 2.444 ^a	$14.709 \pm 3.409^{\mathrm{a}}$		
Weight gain (g)	4.722 ± 2.287 ^c	6.913 ± 2.109^b	11.03 ± 2.29^a	11.067 ± 3.27 ^a		
Weight gain %	146.41 ± 69.03 ^d	153.90 ± 33.39 ^c	277.50 ± 46.46^b	305.45 ± 96.39^a		
SGR (%/day)	1.164 ± 0.403 ^a	$1.234 \pm 0.17^{\text{a}}$	$1.765 \pm 0.159^{\text{a}}$	1.841 ± 0.37 ^a		
Survival rate $(\%)$	93.3 ± 2.87 ^d	90.0 ± 8.6 ^c	95.0 ± 5.0^b	$96.67 \pm 2.89^{\text{a}}$		

a,b,c and d Means in the same raw with superscript are significant different at (p≤0.05

Table(4 .4): Illustrate Water quality parameters as measured in experimental different level of Pumpkin seed meal for 11 weeks.

a,b,c Means in the same raw with superscript are significanty different at (p≤0.05

Figure: (1) Food Conversion Ratio for O.nilotcus fed diets containing different levels of Pumpkin seed meal for 11 weeks.

Feed intake

Figure: (2) Feed intake for O.nilotcus fed diets containing different levels of Pumpkin seed meal for 11 weeks.

CHAPTER FIVE

DISCUSSION

Fish fed 40% pumpkin seed meal level showed the highest % AWG $(305.45 \pm 96.39\%)$ and SGR (1.84 ± 0.37) among fish fed different level of pumpkin seed meal containing diets (Table 4.2). There were significant differences between the % AWG and SGR of fish in all diets. Nonetheless, increase pumpkin seed meal level in diets resulted in positive growth response in fish. T0 (control) showed lowest AWG and SGR these result with the same line with **(Davies et al. 1989)** reported that only 15% rapeseed meal could effectively replace FM in *O. mossambicus* diets, while higher levels resulted in poor growth due to the high content of glucosinolate (antinutrient) in rapeseed. There were significant differences between the FCR of fish in all treatments compared to the T0 diet. It has been reported that plant protein-based diets can lead to lower nitrogen retention in salmonids as these diets contain lower digestible energy and suboptimal amino acid profile critical for muscle growth **(Cheng et al 2003).**

Furthermore, effect of pumpkin seed meal level supplementation on proximate composition level was investigated. It was presumed that diets with 20% and 40% pumpkin seed meal contained higher protein level (Table 4.2). Contrary to the results in this study, fish fed 10% pumpkin seed meal, which was expected to show lowest protein level, actually showed significantly lower protein content than in fish fed other diets.

Fish fed 10% pumpkin seed meal showed the numerically highest lipid level, although there were significant differences in lipid levels among diets. It has been reported that replacement of fishmeal with plant protein sources could increase lipogenic enzyme activities in sea bass (*Dicentrarchus labrax*),

32

which could lead to increased lipid and gross energy content in the whole body of fish at constant level of protein **(Kaushik et al 2004).**

Fish fed 10% pumpkin seed meal showed numerically highest feed intake compared to fish fed other diets. Meanwhile, fish fed 40% pumpkin seed meal showed significantly lower than fish fed other diets.

The physical and chemical water characteristics were studied during this study showed the variation in PH, D.O, Temperature, NO2, $NH₃/NH₄$, PO_4 and NO_3 between the different treatments.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion:

Fish fed 40% pumpkin seed meal exhibited numerically highest growth performance and feed utilization efficiency. Moreover, diets with 10 to 20% and 40% pumpkin seed meal were comparable to the positive control diet, as these were found to be efficiently utilized by *O. niloticus* fingerlings as well. Therefore, pumpkin seed meal may pose as a potential ingredient for fishmeal replacement in *O. niloticus* feeds.

6.2 Recommendations

Based on the finding from the present study we recommended that:

- Further research on the effects of pumpkin seed meal on amino acid profiles of both diets and fish carcass is recommended.
- Further research on the effects of pumpkin seed meal on fatty acids profiles of both diets and fish carcass is recommended.
- Pumpkin seed meal may also be tapped as these contain higher amounts of protein, and hence part ial replacement for fishmeal.
- When more efficient means of defatting seeds is available, higher levels of seed may be investigated to see whether or not it can yield better growth performance in fish.

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