بسم الله الرحمن الرحيم

# *Replacement of GUM ARABIC for Tilapia Fingerlings Based On Plant Source Diet Inclusion*

**Submitted by**

***Naima rabiee Mohamed***

***Rbab Mohamed alfaddul***

For the Degree

*Barchlor of Science*
*(FISHERIES and wildlife)*

***Department of Fisheries and Wildlife Science***

***College of Animal Production Science and Technology***

***Sudan University of Science and Technology***

**ADVISOR**
**Asaad Hassan Widaa Mohamed. PhD.**

***OCTOBER, 2015***

# DEDICATION

***To our parents***

***You're*** *the thought in OUR dreams. You're the vision in OUR eyes. You're the sound in OUR ears. You're the words in OUR mouth. You're everything WE need. You're everything WE want. You're everything that makes us, US.*

***To our siblings***

*For the love, care, endless support, patience, and for all the sacrifices;*
*sentiment reciprocated with deep sense of gratitude and manifestation,*
*With petals of roses, Palm full of holy water, Light of sunshine, Fragrance of Flowers and Grass with the dew;*

*This humble piece of work is wholeheartedly dedicated*

# *ACKNOWLEDGEMENT*

*At this very moment of accomplishment, first of all and as We always have until our last breathe, Very special thanks to ALMIGHTY ALLAH, the merciful who gave us the opportunity to complete this task; and offer our humblest thanks from the deepest core of our hearts to the HOLY PROPHET MUHAMMAD '(Peace Be Upon Him)' who exists eternally as an embodiment of knowledge and sages for the whole mankind.*

*At this moment of accomplishment, I pay homage to my guide; Dr. ASAAD HASSAN WIDAA MOHAMED. This work would not have been possible without his guidance, support and encouragement. Under his guidance, we successfully overcome many difficulties and learned a lot. We can't forget his hard times. Despite of his busy schedule, he used to be always there and review my papers progress, give his valuable suggestions and recommendations, invaluable advice on diverse topics whenever he received our requests and wherever he was. His consistently prompt response on urgent matters and strong encouragement were of vital importance to the completion of our study. His unflinching courage and conviction will always inspire us, and we wish to continue to work with his noble thoughts through our future work yet to come. This Thesis is a small tribute to an exceptional man from a student still anxious to learn from him.*

*Also, we gratefully acknowledge MR. FAWZI ALI MOHAMED, lecturer at department of fisheries and wildlife for his encouragement and helpful attention which have provided good and smooth basis for our study.*

# *List of contents*

# *List of Figures*

# *List of Tables*

# *Abstract*

***Nile tilapia most dominant cultured tilapia species in Sudan and have contributed significantly to the aquaculture development of the country. A 6 week trial was conducted to study the growth of this species under three different culture regimes (two levels of feeding regimen in hapa-in-pond). Mixed sex fingerlings of average weight 3.90 ± 0.21 g were stocked in hapa-in-pond at a rate of 5fish/hapa. Fingerlings were fed a 25.15% crude protein fish diet. A comparison of growth performance of fish under the three feeding regimes with varying concentration of Gum Arabic incorporated into the diet in terms of weight gain was in the following order; (3% GA) > (6% GA) > and (9% GA). There were significant differences in final mean weights mean daily weight gain, specific growth rate and mean weight gain between the GA regimes yet better in lower percentage treatment. Although the net yield was high in O. niloticus under the control treatment, survival was relatively high among O. niloticus under the 3% GA treatment. A high value of fish crop was realized in O. niloticus under the control condition with no GA, however, did vary significantly from each other. In general, although O. niloticus showed superior growth with an appreciable feed conversion rate,***



Keywords: *Oreochromis niloticus*, Gum Arabic, Hapa in pond, feed conversion ratio, Survival.

# Chapter One - INTRODUCTION

The demand for compound fish feed was estimated to be 29.3 million tons in 2008 and is expected to grow along with increased global aquaculture production **(FAO, 2011)**. The compound fish feed production has grown at an average rate of 10.9 percent/ year since 1995 **(FAO, 2011)**. In order to reduce the pressure on fish meal in the gradually increasing in aquaculture industry, alternative feed ingredients from the plant, microbial and other animal sources have been a prioritized field of research from last one decade.

Fish feeding costs amount to 70% of the operational cost of fish farming. The shortage in global production of fish meal, which is the major traditional animal protein source combine with its increased demand in feeds for livestock, poultry and other husbandry animals, is likely to decrease the dependence on it as a single protein source in aqua feeds **(El-Sayed, 1999).** Fish meal is considered the most advantageous animal protein ingredient in aquaculture feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polygenic fatty acids **(Hardy and Tacon, 2002)**. Therefore, fisheries scientists have made a multiple efforts to partially or totally replace fish meal with less expensive and locally available protein sources, such as legumes **(De Silva *et al*., 1988),** lupine seed meal **(De La Higuera *et al*., 1988),** soybean meal (SBM) **(El-Saidy and Gaber, 1997; Eric *et al*., 2000; Carter and Hauler, 2000),** cottonseed meal **(El-Sayed, 1999; El-Saidy and Gaber, 2004),** cluster bean meal **(El-Saidy *et al*., 2005),** faba bean meal **(Deyab *et al*., 2006)** and cow pea seed meal **(Deyab and Aml Saad, 2008)** sesame seed meal **(Deyab *et al*., 2009)** which were reported to be suitable as a partial replacement for fish meal.

Many different plants-protein sources have been examined, including plant-protein meals and plant protein concentrates **(Lim *et al*., 2008)**. Inclusion of feedstuffs with relatively high levels of carbohydrate in formulating fish feed is preferred in view of its protein-sparing action that may make the diet most cost effective **(Hidalgo *et al*., 1993)**.

**Bhosale *et al*. (2010)** reported that an increased use of plant protein supplements in fish feed can reduce the cost of fish meal. The research has focused on utilizing less expensive and readily available resources to replace fish meal, without reducing the nutritional quality of feed **(Mahboob and Sheri, 1997).** There is less information available on the use of plant as an alternative source for preparation of

Fish feeds.

Gum Arabic (GA), is a natural composite polysaccharide derived from exudates of *Acacia senegal* and *Acacia seyal* trees. GA consists of mainly three fractions **(Randall *et al*., 1988; Idris *et al*., 1998):** the major one is a highly branched polysaccharide (MW=3x105) consisting of b-(1-3) galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid (found in nature as magnesium, potassium, and calcium salt) **(Dror *et al*., 2006).** A smaller fraction (~10 wt % of the total) has a higher molecular weight (~1x106 g/mol) called arabinogalactan–protein complex (GAGP – Gum arabic glycoprotein) in which arabinogalactan chains are covalently linked to a protein chain through serine and hydroxyproline groups. The attached arabinogalactan in the complex contains ~13% (by mole) glucoronic acid **(Goodrum *et al*., 2000).** The smallest fraction (~1% of the total) having the highest protein content (~50 wt %) is a glycoprotein which differs in its amino acids composition from that of the GAGP complex.

The major amino acids present in the protein of AG and AGP are hydroxyproline, serine and proline, whereas in GP, aspartic acid is the most abundant **(Islam *et al*., 1997).** The purpose of this study therefore to examine Gum Arabic plant powder as a partial replacement for fishmeal in diets for *O.niloticus* fingerlings and to utilize the findings to develop a cost effective ration for fish culture in Sudan.

# Chapter Two – LITERATURE REVIEW

## *Gum Arabic*

Gum arabic, known as acacia gum, *chaar gund*, *char goond*, or *meska*, is a natural gum made of hardened sap taken from two species of the acacia tree; *Senegalia senegal* and *Vachellia seyal*. The gum is harvested commercially from wild trees throughout the Sahel from Senegal to Somalia, although it has been historically cultivated in Arabia and West Asia. It is a complex mixture of glycoprotein and polysaccharides. It was historically the source of the sugars arabinose and ribose, both of which were first discovered and extracted from it (**Renard *et al*., 2006; Calame, *et al*., 2008).**

Gum Arabic as Prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal since birth and beneficial to the digestive system, body's immunity, disposal of poisons, fats and excreta (**Da Silva *et al.*, 2013)**. Besides it negates the effects of harmful bacteria thus protecting from the diseases, cancer, diabetes and obesity. It is pointed out that the gum-Arabic, extracted from *Acacia senegal* trees which is more common in Sudan than anywhere in the world, is one of the natural sources of the prebiotics similar to breast milk and the processed insulin (**Gerstenzang *et al*.,2007; Calame, *et al*.,2008**).

Gum Arabic was evaluated for acceptable daily intake for man by the Joint FAO/WHO Expert Committee on Food Additives since 1969 **(FAO/WHO, 1969)**; however, Sudanese people in Western Sudan had been using it for long time without limitations. It is indigestible to both humans and animals, not degraded in the intestine, but fermented in the colon to give short-chain fatty acids, leading to a large range of possible health benefits **(Phillips and Phillips, 2011)**.

Several epidemiological studies suggest that a high intake of dietary fiber, including GA, is associated with beneficial effects on fat metabolism **(Ali *et al.*, 2009).** Dietary fiber promotes satiation and satiety, alter glycogenic index, affects gastric emptying, gut hormone secretion and thus helps to manage weight. Leptin promotes weight loss by two different mechanisms. It reduces appetite, and thus food intake, and at the same time increases energy expenditure. Also dietary fiber was inversely associated with Leptin level in young Japanese adults **(Kuroda *et al.*, 2010)**. In addition to that, a study has shown that GA inhibits intestinal glucose absorption via interaction with membrane abundance of SGLT1 in mice **(Kuroda *et al.*, 2010)** GA significantly blunted the increase in body weight, fasting plasma glucose and fasting insulin concentrations during high fat diet.

## ***Nile tilapia***

Tilapias 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 metric tons in 1990 to 1,505,804 metric tons in 2002, representing about 6% of total farmed finfish in 2002 **(FAO, 2004)**.

Nutrition is the most expensive component in 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. The major challenge facing tilapia nutritionists in developing countries is the development of commercial, cost effective tilapia feeds using locally available, cheap and unconventional resources.

This review throws some light on protein nutrition of farmed tilapia with emphasis on the use of unconventional, unutilized protein sources. Protein requirements of tilapia have been extensively studied using dose-response procedures. In this regard, semi-purified test diets containing casein, casein/gelatin mixtures or casein/amino acid mixtures as protein sources or using practical diets in which animal and/or plant ingredients served as dietary protein sources have been widely used. The results of many studies are questionable, because they e.g. were conducted indoor, short-termed, may not be directly applied in field trials, and relied mainly on casein (which is deficient in the essential amino acid (EAA) arginine) as a sole dietary protein. Casein/gelatin-based diets were found to be utilized more efficiently than casein/amino acid (AA) diets **(El-Sayed, 1989)**.

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. For example, 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 (**Winfree and Stickney, 1981; Jauncey and Ross, 1982; Siddiqui *et al*., 1988; El-Sayed and Teshima, 1992)**. For tilapia juveniles, the protein requirement ranges from 30-40%, while adult tilapia requires 20-30% dietary protein for optimum performance. On the other hand, tilapia broodstock require 35-45% dietary protein for optimum reproduction, spawning efficiency, and larval growth and survival **(Gunasekera *et al*., 1996; Siddiqui *et al*., 1998; El-Sayed *et al*., 2003)**.

Since purified and semi-purified protein sources are not recommended for tilapia under commercial culture conditions, other conventional and unconventional, locally available dietary protein sources should be considered. Research has evaluated many such sources for different species of tilapia, with varying results.

Therefore, it is appropriate to highlight these protein sources for tilapia, with emphasis on the sources that have economic potential and are locally available, especially in developing countries. A comprehensive review of the possible alternative protein sources for farmed tilapia has been reported by **El-Sayed (1999)**.

## *Animal protein sources*

### ***Fish meal***

Fish meal (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 in global 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, 1993).** Many developing countries have realized that, in the long-run, they will be unable to afford FM as a major protein source in aqua feeds. Therefore, many attempts have been made to partially or totally replace FM with less expensive, locally available protein sources. A wide variety of unconventional protein sources, including animal proteins, plant Proteins, single-cell proteins and industrial and agricultural wastes have been evaluated with respect for their utility in farmed tilapia feeds.

Some sources were found cost effective, while others were not. The following evaluation of alternative protein sources will provide farmers and nutritionists with information on their advantages and disadvantages of such feed ingredients as well as their proper inclusion levels in tilapia feeds.

## *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 these by-products for tilapia feeds. 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 to 75% fish silage can be successfully incorporated in tilapia feed, depending on fish species and size, silage source, and diet composition **(Fagbenro, 1994; Fagbenro and Jauncey, 1994; Fagbenro *et al*., 1994)**.

It is evident that fish silage has potential as a protein source for tilapia. The quality of fish silage is affected by the fermentation and/or silaging methods. For example, diets containing formic acid-preserved fish silage produce reduced growth performance of tilapia, presumably due to acidity of the diet and high a proportion of free amino acids in the fish silage. It has been suggested that acidity reduces diet acceptance and affects protease activity in fish guts **(Hardy *et al*., 1983)**, while free amino acids may depress fish appetite **(Wilson *et al*., 1984)**. Further, shrimp meal has also been successfully used as a protein source for tilapia. Blue tilapia (*O. aureus*), and Nile tilapia utilized shrimp head meal at 15% and up to 60% of the diet without adverse effects on their performance **(Toledo *et al*., 1987; Nwanna and Daramola, 2000)**.

Moreover, **Mansour (1998) and El-Sayed (1998)** reported that shrimp meal can replace FM in red tilapia (*O. niloticus x O. hornorum*) and Nile tilapia diets, at 50% and 100%, respectively, without significant retardation in weight gain and feed efficiency.

## *Terrestrial animal by-products*

Terrestrial animal by-products including poultry by-product meal (PBM), blood meal (BM), hydrolyzed feather meal (HFM) and meat and bone meal (MBM) have been widely used as protein sources for tilapia, due to their high protein content and good EAA profiles **(Tacon, 1993).** However, they may be deficient in one or more of the EAA. The most limiting EAAs in these by-products are lysine (in PBM, HFM), isoleocine (BM) and methionine (MBM, BM, HFM) **(NRC, 1983; Tacon and Jackson, 1985)**. If these by-products are included in the feed at the proper ratios, the EAA deficiencies can be overcome and the quality of such diets is likely to improve **(Tacon *et al*., 1983; Davies *et al*., 1989)**. **Tacon *et al*. (1983)** found that hexane extracted MBM or MBM:BM (4:1) supplemented with methionine successfully replaced up to 50% of FM protein in Nile tilapia fry diets. Furthermore, **Davies *et al*. (1989)** found that optimum MBM/BM ratios could replace up to 75% of FM in diets fed to *O. mossambicus* fry.

They also found that diets containing MBM or high MBM/BM ratios (3:1 and 2:3) were superior to FM even at a 100% substitution level. Cost-benefit analyses indicated that these sources can be used as single dietary protein sources for Nile tilapia **(El-Sayed, 1998)**. On the contrary, BM and HFM are not efficiently utilized by tilapia due to low digestibility and poor EAA profiles **(Viola and Zohar, 1984; Davies *et al*., 1989; Bishop *et al*., 1995)**.

Terrestrial animal by-product silage has been successfully used as a protein source for tilapia. **Belal *et al*. (1995)** fed *O. niloticus* fingerlings (10.8 g) test 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 up to 20% level were similar to that of fish fed a FM based diet.

## *Plant protein sources*

## *Oilseed plants*

### ***Soybean Meal***

Soybean meal (SBM) contains the highest plant protein content and has the best EAA profile, but it is deficient in sulfur-containing amino acids (Met, Lys, Cys), and contains endogenous ant nutrients, including protease (trypsin) inhibitor, phytohaemagglutinin and anti-vitamins. Some of the factors can be destroyed or inactivated during thermal processing **(Tacon, 1993).** SBM can be used as a total or partial protein source for farmed tilapia, depending on fish species, size, dietary protein level, SBM source and processing methods. For example, processed, solvent extracted SBM, with or without Met supplementation, successfully replaced up to 75% of FM in the diet of Nile tilapia fry **(Tacon *et al*., 1983)**, *O. mossambicus* **(Jackson *et al*., 1982)** and 67% in the case of tilapia hybrids **(Shiau *et al*., 1989)**.

Supplementing SBM with the deficient EAA did not improve fish growth, and therefore was proven unnecessary **(Teshima and Kanazawa, 1988)**. It should be realized that the quality of SBM (and other plant protein sources) for tilapia depends on the processing methods. SBM germination **(Wassef *et al*., 1988)** and heating reduce, but may not eliminate the activity of protease inhibitors.

**El-Sayed *et al*. (2000)** found that full fat SBM contained traces of protease inhibitors even after thermal treatment (at 200$^{o}$C for 10 min) or soaking for 3 days, leading to an increase in trypsin secretion (to compensate for the reduced activity) in Nile tilapia.

***Cottonseed meal/cake***

Cottonseed meal (CSM) 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, 1983)**. However, it is deficient in some EAA such as Cys, Lys and Met in addition to its high content of gossypol (a phenolic antinutrient) that may limit the use of CSM in tilapia feeds. Results on the use of CSM and CSC (cottonseed cake) indicated that replacement of more traditional protein sources at between 50 and 100% can be effective in tilapia feed, depending on CSM source, processing methods and fish species and size.

***Other oilseed by-products***

Few studies have considered other oilseed by-products, such as groundnut, sunflower, rapeseeds, sesame seeds, copra, macadamia, cocoa cake and palm kernel, despite their good potential as protein sources for tilapia. **Jackson *et al*. (1982)** found that rapeseed meal could effectively replace up to 75% of FM protein in *O. mossambicus* diets. On the other hand, **Davies *et al*. (1990)** found that only 15% rapeseed meal could effectively replace FM/SBM in *O.mossambicus* diets, while higher levels resulted in poor growth and feed efficiency due to the high content of glucosinolate (antinutrient) in rapeseed. Similar results were reported with respect to the use of macadamia press cake (MC) as a protein source for tilapia **(Fagbenro, 1993; Balogun and Fagbenro, 1995)**.

***Aquatic plants***

Several studies have been conducted on the use of aquatic plants in tilapia feeds. Among these plants, the duckweed (family: *Lemnaceae*) is the most promising. Duckweed can be an excellent food source for tilapia, due to its good protein content (35-45%) and amino acid and mineral profiles. It can be cultivated easily, yielding 10-50 dry metric tons/ha/year **(Leng *et al*., 1995)**.

Duckweed can be used as a single food source for farmed tilapia **(Fasakin *et al*., 1999)**. Furthermore, **Skillicorn *et al*., (1993)** reported that when duckweed was used as a single nutritional input for tilapia in earthen ponds, fish production reached 7.5 metric tons/ha/yr. Dry duckweed can also replace up to 50% of the commercial feed without adverse effects on fish performance **(Arrivillaga, 1994; Essa, 1997).**

Other aquatic plants including *Azolla pinnata* (a freshwater fern having a symbiotic Relationship with nitrogen fixing cyanobacteria *Anabaena azollae*). *Hydrodictyon reticulatum*, coontail (*Ceratophyllum demersum*), chuut-nuu (*Eleocharis ochrostachys*) and *Potamogetonvgramineous* can be used as a partial replacement of standard protein for different tilapia species **(Appler, 1985; Chiayvareesajja *et al*., 1990; Klinnavee *et al*., 1990; El-Sayed, 1992)**. However, these sources should be carefully looked at, since some other aquatic plants such as *Elodea trifoliate* and *Muyriophyllum spicatum* have been reported to reduce tilapia performance.

***Grain legumes***

Many leguminous or cereal plants and by-products can be used as partial protein sources for tilapia. Among these, *leucaena* leaf meal (LLM, 30% crude protein), brewery wastes, corn products (gluten, gluten feed, distiller's grain, co-products), cassava leaf meal, green gram legume, lima bean and leaf protein concentrates are of prime importance.

However, most of leguminous or cereal plants are deficient in certain EAA (e.g. Arg, Thr, Iso, His, Met are deficient in LLM) and may contain ant nutrients such as mimosine (a toxic non-protein amino acid) found in LLM **(Lim and Dominy, 1991)**. Proper processing of these sources may improve their quality for tilapia.

***Single-cell proteins***

Single cell proteins (SCP) such as unicellular algae, fungi, bacteria, cyanobacteria, and yeast are traditionally used as natural food for tilapia in semi-intensive systems. In intensive pond farming systems, SCP can also be used if a carbon source (such as wheat bran, rice bran and cellulose) is sprayed on the surface of pond water with continuous aeration. At the optimum carbon: nitrogen ratio (15:1), bacterial growth will increase **(Chamberlain and Hopkins, 1994)** and consume the carbon source as energy and reduce ammonia concentration through nitrification, while the fish feed on produced bacteria. By this way, a cheap carbon and nitrogen sources can partially replace expensive commercial protein sources in tilapia feeds.

***Protein digestibility***

One major problem associated with the determination of dietary protein requirements of tilapia is the interchangeable and inconsistent use of terminology. Many authors often use ME and DE values interchangeably, and uses varying energy values for the same ingredient under the same terminology. In addition, energy values reported for other fish species have been widely used in the preparation of tilapia diets **(El-Sayed and Teshima, 1991)**. The digestibility coefficients of a number of ingredients commonly used as a protein sources for tilapia have been determined. It is, therefore, more appropriate to use these reported values for tilapia instead of using ME or DE values reported for other fish species.

***Improving protein quality***

As mentioned earlier, many of the protein sources in tilapia feeds are deficient in certain EAA. The supplementation of these EAA into the diets has been a common practice. However, it was found that the utilization of many protein sources in tilapia feeds may be limited by dietary minerals (such as phosphorus and zinc), rather than the deficient EAA. This means that the inclusion of dietary EAA may not be necessary if the diet contains proper levels of certain minerals. For example, the inclusion of dietary phosphorus source to SBM-based diet may meet the requirement for deficient EAA (Methionine).

**Viola *et al*. (1986, 1988)** reported that the non inclusion of the deficient EAA to SBM-based diet did not result in any growth retardation, while SBM supplemented with 3% dicalcium phosphate (DCP) and oil completely replaced FM without any adverse effects on fish growth. The non-necessity of EAA supplementation has also been reported with sesame seeds **(El-Sayed, 1987) and CSM (El-Sayed, 1990)**. Sesame seeds are deficient in Lys and zinc. The supplementation of either Lys or zinc significantly improved the growth and survival of *T. zillii* **(El-Sayed, 1987)**. Once again, Lys or zinc may meet the requirement of one another, supporting the argument that certain minerals rather than EAA deficiency may be the limiting factor in sesame seeds. Adopting this approach may improve the protein quality and reduce the cost of the diets.

***Phytase supplementation***

Many plant protein sources contain high levels of phytic acid, which binds with divalent minerals such as Ca, P, Zn, Mn, Mg, and Fe to form water-insoluble salts, rendering the minerals unavailable. When these plants are used as the primary source of protein in a tilapia feed, higher supplementary mineral levels may be required, particularly if the culture water is deficient in one or more of the required minerals.

The inclusion of bacterial Phytase in tilapia diets can also be an effective tool in reducing phytic acid activity and improving the utilization of plant protein sources. Phytase may also reduce the effect of anti nutritional factors, protect amino acids from degradation, and decrease leaching of water soluble components **(Riche *et al*., 2001; Heindl *et al*., 2004)**. Many recent studies indicated that the addition of Phytase into tilapia diets has improved growth rates, digestibility and utilization of dietary protein phosphorous **(Riche *et al*., 2001; Heindl *et al*., 2004; Liebert and Portz, 2004; Phromkunthong *et al*., 2004)**. These studies demonstrated that about 750-1000 Phytase unit/kg feed were required for optimum performance, depending on dietary plant protein: animal protein ratios and mineral contents of the diets.

***Economic evaluation of protein sources***

Some of the protein sources described above may result in a significant reduction in fish performance, but still they can be more cost/effective than standard, expensive proteins (such as FM). Therefore, economic evaluation of such protein sources for tilapia is necessary. However, only a few studies have considered both economic and biological evaluation of dietary protein sources for tilapia. These studies demonstrated that sources like CSM **(El-Sayed, 1990),** corn gluten feed and meal **(Wu *et al*., 1995)** animal by-product meal **(Rodriguez-Serna *et al*., 1996; El- Sayed, 1998)** and brewery waste **(Oduro-Boateng and Bart-Plange, 1988**) can be used as total fishmeal replacers for tilapia despite they produced lower biological performance. Of course, more work in needed alongside this line.

# Chapter Three – MATERIAL AND METHODS

## *Diet formulation and preparation*

Feedstuffs were prepared at fish lab at college of Animal production Science and Technology, Sudan University of Science and Technology - Khartoum, and were separately milled to small particle size (< 250 µm) using pulverizing machine. The nitrogenous diet was formulated form ingredients as followed in (**Table I**).

Prior to storage, the diet were analyzed for proximate composition according to standard methods **AOAC (1990)**. Crude protein was determined using a Kjeldahl Analyser after digestion with concentrated $H_2SO_4$ in a digester. Crude lipid was estimated by extracting in chloroform: methanol (2:1) using a Soxhlet extraction unit.

**Table I: Ingredients Composition of the Experimental Diet**

| *Ingredients* | *Control* | *Treatment 1* | *Treatment2* | *Treatment3* |
|---|---|---|---|---|
| ***Fish meal*** | ***30*** | ***30*** | ***30*** | ***30*** |
| ***Ground cake*** | ***21*** | ***21*** | ***21*** | ***21*** |
| ***Wheat bran*** | ***20*** | ***20*** | ***20*** | ***20*** |
| ***Bread floor*** | ***10*** | ***10*** | ***10*** | ***9*** |
| ***Starch*** | ***10*** | ***7*** | ***4*** | ***2*** |
| ***Veg.oil*** | ***5*** | ***5*** | ***5*** | ***5*** |
| ***Min.mix*** | ***4*** | ***4*** | ***4*** | ***4*** |
| ***Gum Arabic*** | ***0*** | ***3*** | ***6*** | ***9*** |
| ***Total Sum*** | ***100*** | ***100*** | ***100*** | ***100*** |

Crude fiber was determined using a Fibretec System 1020 Hot Extractor and ash content was determined by igniting at 550°C in a muffle furnace for 12 hours.

**Table II: Proximate Composition of the Experimental Diets**

| *Treatment* | *Moisture* | *D. matter* | *Ash* | *C. fiber* | *C. fat* | *C. protein* | *N.F.E* |
|---|---|---|---|---|---|---|---|
| ***Control*** | ***3*** | ***97*** | ***14.5*** | ***3.90*** | ***3.03*** | ***25.5*** | ***50.43*** |
| ***Feed1*** | ***3.5*** | ***96.5*** | ***14.5*** | ***3.66*** | ***2.8*** | ***23.43*** | ***51.61*** |
| ***Feed2*** | ***4.5*** | ***95.5*** | ***11.5*** | ***3.6*** | ***2.81*** | ***23.17*** | ***54.43*** |
| ***Feed3*** | ***4.5*** | ***95.5*** | ***13*** | ***3.55*** | ***2.78*** | ***20.12*** | ***56.05*** |

### *Experimental system and animals*

*O. niloticus* fingerlings were obtained from a reputable fish farm in Elsilit province, Khartoum State, Sudan, and acclimated for 14 days in Hapa nets (75 x 75 x 100 cm). Two-third of each hapa was filled with water. The fish were being fed on a commercial pelletized diet (25.15% crude protein). Four treatments namely, daily feeding frequencies 9% of control (FF1), 3% as (FF2), 6% as (FF3), and 9% as (FF4), respectively.

After acclimation, five (5) *O. niloticus* (3.40g±0.04) fingerlings were randomly stocked in each hapa net measuring (75 x 75 x 100 cm) with approximately 400 liters of fresh water capacity.

Feeding commenced a day after stocking and lasted 40 days. The fish were hand-fed to apparent satiation at different feeding frequencies with 9% from its body weight. All fish were removed from each hapa net every ten days and batch weighed. Mortality was monitored daily and recorded. Growth performance and nutrient utilization indices were determined as final fish weight (g), survival (%), specific growth rate (SGR, % $day^{-1}$) and feed conversion ratio (FCR). Growth parameters were calculated as follows:

% weight gain (% $fish^{-1}$) = [(final wt – initial wt)/initial wt.)] X 100

Weight gain (g) = (final wt – initial wt)

Specific growth rate (% $day^{-1}$) = [(ln final wt – ln initial wt)/initial wt] X 100

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

Water temperature and dissolved oxygen were measured as well as pH which monitored weekly before the sampling intervals.

***Statistical Analysis***

Data were tested for uniformity in variance and normality of distribution before they were analyzed using Analysis of Variance (ANOVA) at P = 0.05 to determine significant differences among treatments. Once significance was detected, data were subjected to post hoc analysis, specifically, Turkey's Test.

# Chapter Four - RESULTS

Trends in growth rate among treatments were clearly differentiated by the first sampling. Growth was gradually increased in all hapas during the entire culture period indicated that fish after 40 days are still growing, the steady increase in growth during the culture period is an indication also that the maximum performance in growth was not achieved yet. The result suggested that 6% GA feeding alone may be enough to support the high growth rate of the fish.

Water quality parameters during the experimental period were as follows: pH 7.4-8.2; dissolved oxygen 5.0-6.0 mg $L^{-1}$ and temperature 26-32ºC. Mean temperature, pH and dissolved oxygen levels were not affected by feeding scheme and very well within the acceptable limits for fish growth and health (**Boyd, 1979**).

After six weeks of the feeding trial, *O. niloticus* fingerlings in the 6% gum arabic inclusion group significantly exhibited the highest mean weight gain and consistently sustained the highest values until the termination of the experiment (**Table II**). In contrast, *O. niloticus* fed continuously without application of gum arabic powder (control group) exhibited statistically lowest mean weight gain with those offered the 3% and 9% gum arabic powder inclusion feeding regimes.

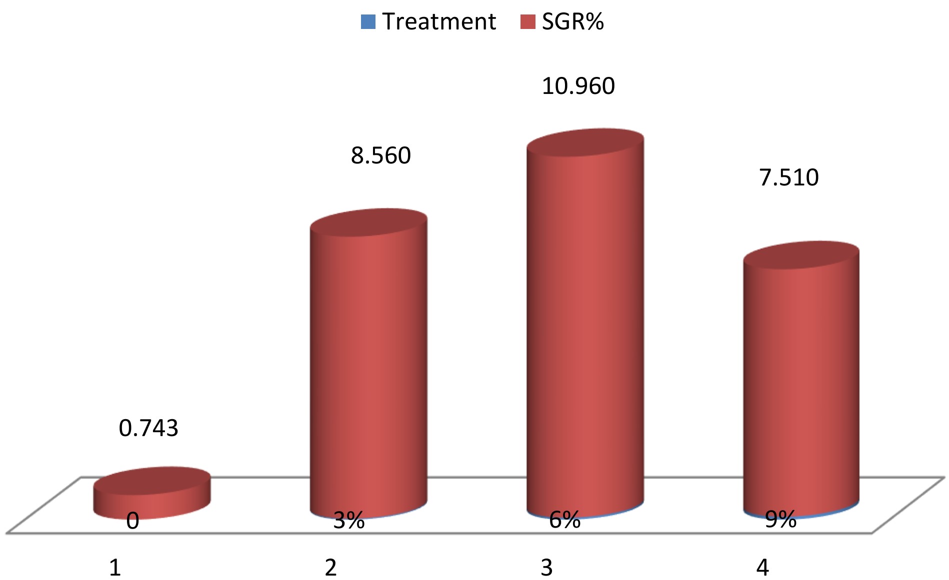
SGR of *O. niloticus* fed continuously without application of gum arabic powder feeding regime exhibited significantly lower specific growth rate (SGR) than those fed with 3%, 6% and 9% gum arabic powder inclusion feeding regimes (**Table III**) (**Figure I**).

**Table (III): The effects of varying Gum arabic powder inclusion on growth performance indices and survival of Tilapia O. *niloticus* fingerlings, (P<0.05).**

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | ***Control*** | ***3%GA*** | ***6%GA*** | ***9%GA*** |
| Weight gain | *19.17±0.24[c]* | *36.33±5.30[b]* | *52.66±4.12[a]* | *32±01.56[b]* |
| Daily weight gain | *0.48±0.01[c]* | *2.88±0.12[b]* | *4.17±0.04[a]* | *2.35±0.15[b]* |
| % Weight gain | *98.29±4.21[a]* | *38.94±1.25[b]* | *78.32±8.31[c]* | *49.17±2.14[d]* |
| SGR (% day) | *0.74±5.09[a]* | *8.56±4.65[a]* | *10.96±2.08[b]* | *7.51±2.55[b]* |
| FCR | *1.29±0.20[a]* | *5.33±1.05[c]* | *3.18±0.66[b]* | *2.64±1.97[b]* |
| Survival | *40[d]* | *93.33[a]* | *80[b]* | *60[c]* |
| FCE | *0.5±0.15[a]* | *1.44±0.21[b]* | *1.35±0.11[b]* | *1.27±0.16[b]* |
| PER | *0.76±0.22[a]* | *1.42±0.42[b]* | *1.99±0.12[c]* | *1.43±0.35[b]* |

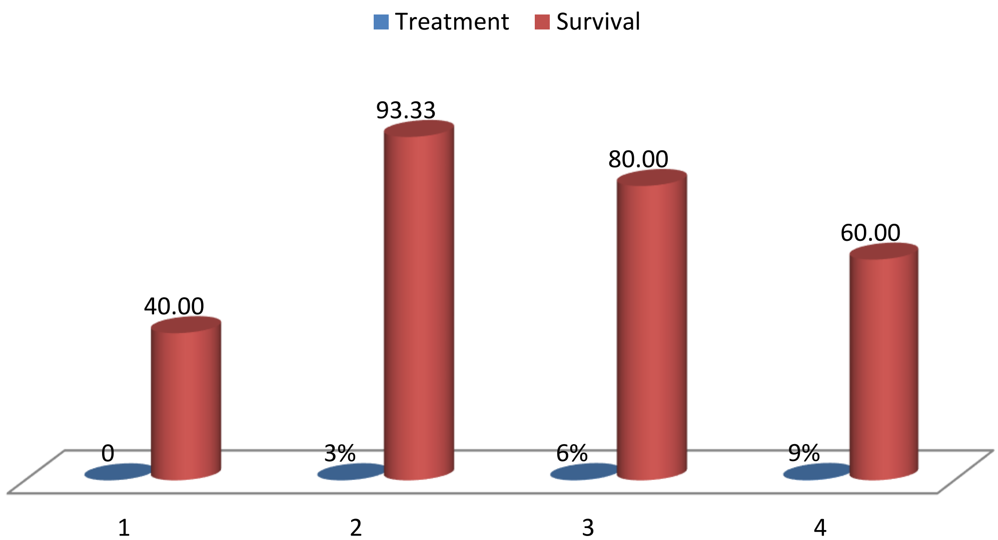
**Mean values (± SEM) with different letters in the same row are significantly different (p < 0.05).**

**Figure I. SGR% of *O. niloticus* subjected to varying feeding inclusions of Gum Arabic**



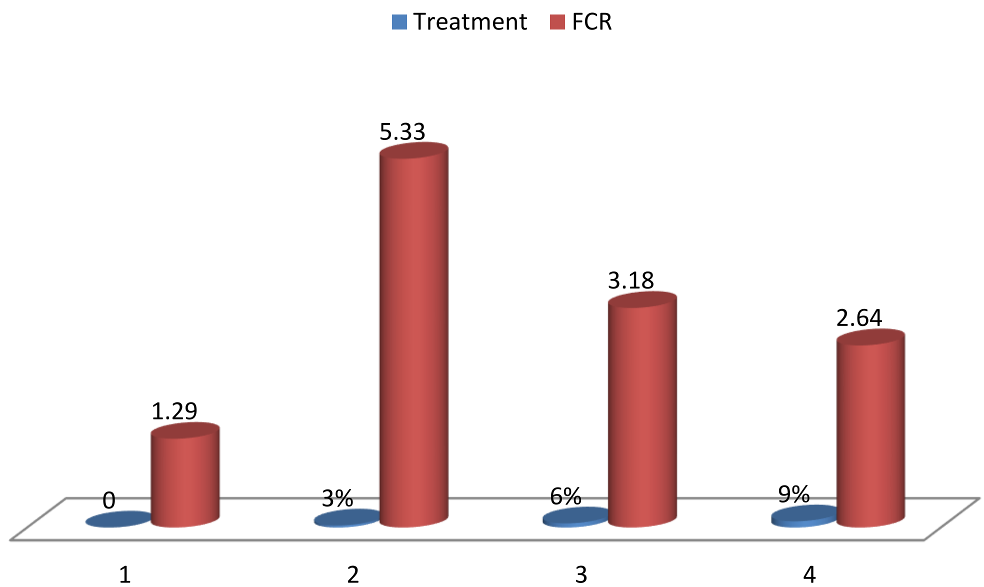

Survival rate were lower due to escapees from the net in groups of control treatment and were statistically significant in all treatments with varying levels of gum arabic powder e.g. 3%, 6% and 9% inclusion respectively in comparison to control treatment. (**Figure II**).

**Figure II. Survival of *O. niloticus* subjected to varying feeding inclusions of Gum Arabic**



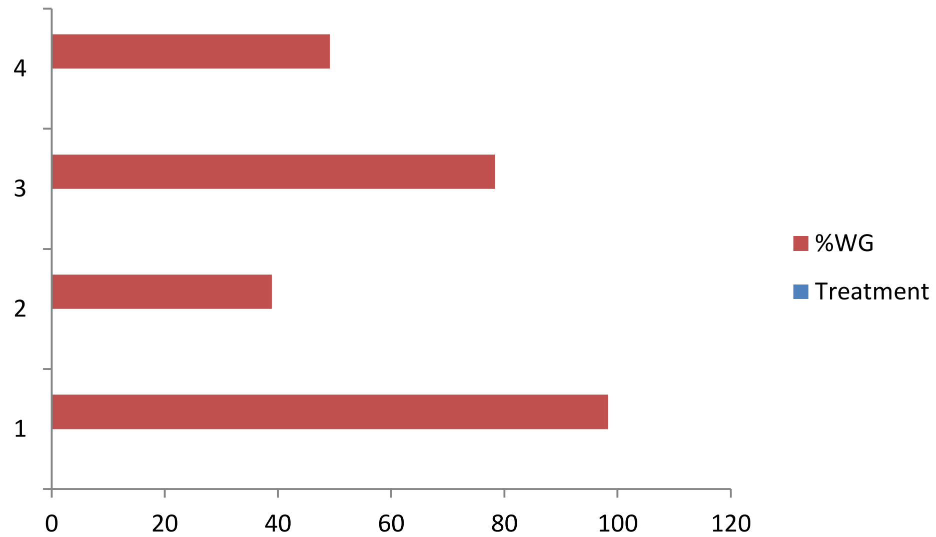

However, values of efficiencies of conversion (i.e. FCR) were significantly the best in *O. niloticus* offered no GA feeding regimen while those of fish offered the 3%, 6% and 9% regimen fed fish exhibited lower values and were statistically differed with each other with no significant differences wherein observed in both 6% and 9% treatments respectively (**Figure III**).

**Figure III. FCR of *O. niloticus* subjected to varying feeding inclusions of Gum Arabic**




The highest growth in terms of weight gain% of *O. niloticus* fingerlings was obtained with control treatment fed no GA incorporated feed, and lowest weight gain % was obtained with 3% GA inclusion treatment. In terms of fish production, gernerally the results showed that different growth parameters such as weight gain and bimass weight gain of tilapia *O. niloticus* fingerlings were affected by GA incorporation in feeds (**Table III**) (**FigureIV**).

**Figure IV. % Weight gain of *O. niloticus* subjected to varying feeding inclusions of Gum Arabic**



# Chapter Five - DISCUSSION

This is the first study carried out to evaluate GA growth promoting activity in fish. In recent years, there have been different studies carried out on GA effects in rats but no one on GA and its effects on biochemical parameters of fish. The use of compounds with immunostimulant and/or antioxidant effects as dietary supplement can improve the innate defence on animals providing resistance to pathogens during periods of high stress. Therefore, the use of immunostimulant is being introduced into fish farming routine procedures as a prophylactic measure (**Kumari and Sahoo, 2006**). Feed formulated with different level of Gum Arabic (3%, 6% and 9%) was used for Tilapia fingerlings rearing under field conditions by feeding fish at different feeding frequencies. During the study period, growth parameters were measured. Survival rate, specific Growth rate, weight gain, daily weight gain, weight gain percentage, food conversion ratio, protein efficiency rate and feed conversion efficiency of fish.

Observation on the growth rate of fishes in various treatments showed that in 40 days culture period, the average weight gain (19.2, 36.3, 52.7 and 32.0) respectively in all treatment. The highest weight gain in ***FFII*** might be due to the fact that the fish had received the small amount of feed at a time and effectively utilized the applied feed effectively converted into muscle.

This study corroborate a similar study by **El-Sayed and Teshima, 1992)** whose reported that 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. For example, 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.

The specific growth rate (SGR), of 8.56, 10.95 and 7.51% per day were found in ***FFI***, ***FFII*** and ***FFIII***, respectively. SGR progressively increased with the decrease in feeding frequency. The significantly highest specific growth rate (SGR) in ***FFII*** might be due to the fact that the fish have utilized effectively the supplied feed taking small amount at a time thrice daily. The survival (%) of fish in different treatments was fairly high ranging from 60 to 93.3% (**Table III**) in all the treatments.

There was significant ($P>0.05$) variation in the survival of fish in different treatments. The survival rate recorded in present study was higher to some extend than the survival rate (82 to 90%) recorded by ***Hussain et al. (1987)***, which might be attributed to the relatively small size of fingerlings stocked in the present study. The lowest i.e. the best FCR (2.64) was observed in treatment ***FFIII*** with three time feeding frequency and the highest i.e. the worst FCR value (5.33) was recorded in ***FFI*** with the feeding frequency of three time a day.

**Gunasekera *et al*., 1996a** reported that for tilapia juveniles, the protein requirement ranges from 30-40%, while adult tilapia requires 20-30% dietary protein for optimum performance. On the other hand, tilapia fingerlings require 35-45% dietary protein for optimum reproduction, spawning efficiency, and larval growth and survival.

The lowest fish production (135.g) was observed in ***FFIII*** which might be due to ineffective feed utilization on bulk ration at a time and resulting decreased feed efficiency. Among the treatments, the highest production was found in ***FFII*** followed by ***FFII*** and ***FFI***, respectively. Considering the overall production and survival rate, the best result was obtained in treatment ***FFIII***.

The crude protein level used in the formulation of the experimental diets for *Oreochromis niloticus* fingerlings (**Table II**) falls within the recommended ranges of crude protein requirement for Tilapia species and satisfied the nutrient requirements for tilapias (***Jauncey, 2000***).

In addition to that, a study has shown that Gum Arabic inhibits intestinal glucose absorption via interaction with membrane abundance of SGLT1 in mice (**Kuroda M, et al ,2010**) Gum Arabic significantly blunted the increase in body weight, fasting plasma glucose and fasting insulin concentrations during high fat diet.

GA administration has a positive effect in growth performance of fish. Probably, these findings underlines that the effect of GA is related to the dosage inclusion and time of administration. So there is still a need for further studies involving a larger number of fish, various concentrations and times of administration of GA and the assessment of additional fish blood parameters to confirm the utility of this substance for fish health then after.

# CHAPTER SIX - CONCLUSION AND RECOMMENDATION

## Conclusion

The results of the present study concluded that application of Gum Arabic has direct effect on the survival rate, specific growth rate, weight gain, daily weight gain, weight gain percentage, food conversion ratio, protein efficiency rate and feed conversion efficiency of Tilapia *O.niloticus* fingerlings under pond culture.

## Recommendations

The present study leads to the following recommendations:

- The Gum Arabic have positive impact on growth parameters of the Tilapia *O. niloticus* fingerlings in all treatments
- Also Gum Arabic may use as alternative protein sources in *Oreochromis niloticus* feeds.
- It is best to feed fish three times feeding frequency in a day.

# Literature Cited


**Appler, H.N. 1985**. Evaluation of *Hydrodictyon reticulatum* as a protein source in feeds for *Oreochromis* (*Tilapia) niloticus* and *Tilapia zillii*. J. Fish Biol. 27: 327-334.

**Arrivillaga, A. 1994**. Effect of substituting prepared feed by the aquatic plant Wolffia sp. (Lemnaceae) on the growth response of tilapia (Oreochromis niloticus) and mojarra paleta (Cichlasoma synspillum) fry. Paper presented at the World Aquaculture Society Annual Meeting, 14-18 Jan. 1994, New Orleans, LA, USA.

**Balogun, A.M. and Fagbenro, O.A. 1995**. Use of macadamia presscake as a protein feedstuff in practical diets for tilapia, Oreochromis niloticus (L.). Aquacult. Res. 26: 371-377.

**Belal, I.E.H., Al-Owaifeir, A. and Al-Dosari, M. 1995**. Replacing fish meal with chicken offal silage in commercial Oreochromis niloticus (L.) feed. Aquacult. Res. 26: 855-858.

**Bhosale, S.V., Bhilave, M. P. And Nadaf, S.B., 2010**. Formulation of fish feed using ingredients from plant sources. Res. J. agric. Sci., 1: 284-287.

**Bishop, C.D., Angus, R.A. and Watts, A. 1995**. The use of feather meal as a replacement for fishmeal in the diet of Oreochromis niloticus fry. Bioresour. Technol. 54: 291-295.

**Chamberlain, G.W. and Hopkins, J.S. 1994**. Reducing water use and feed cost in intensive ponds. World Aquaculture 25 (3): 29-32.

**Da Silva Sandra Tavares, Carolina Araújo dos Santos and Josefina (2013)**: intestinal microbiota relevance to obesity and modulation by prebiotics and probiotics Nutr Hosp.28(4):1039- 1048.

**De La Higuera, M., Garcia-Gallego, M., Sanz, A., Cardenete, G., Suarez, M.D. And Moyano, F.J., 1988**. Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout, Salmo gairdneri. Aquaculture, 71: 37–50.

**De Silva, S.S., Keembiyahetty, C.N. And Gunaskera, R.M., 1988**. Plant ingredient substitutes in Oreochromis niloticus (L.) diets: ingredient digestibility and effect of dietary protein content on digestibility. J. aquat. Trop., 3: 127–138.

**Deyab, M. S. D. E., Mahmoud, S.H., El-Garhy, M. And Tonsy, H.D., 2009**. Nutrition evaluation of sesame seed meal, Sesamum indicum (L) as alternative protein source in diets of juvenile mono-sex Nile tilapia (Oreochromis niloticus). Egypt. J. Aquacul. Biol. Fish., 13: 93–106.

**Deyab, M. S. D. E., Midhat El-Kasheif, A. And Ibrahim, S.A., 2006**. Evaluation of broken faba beans meal, Vicia faba (L.) as a partial or total replacement of fish meal in practical diets of Nile tilapia, Oreochromis niloticus (L.) fingerlings. J. Egypt. Aquacul. Soc., 1:34– 46.

**Deyab, M.S.D.E. And Aml Saad, S., 2008**. Evaluation of cow pea seed meal, Vigna sinensis, as a dietary protein replacer for Nile tilapia, Oreochromis niloticus (L.), fingerlings. J. World aquat. Soc., 39: 636–645.

**Davies, S.J., McConnell, S. and Bateson, R.I. 1990**. Potential of rapeseed meal as an alternative protein source in complete diets for tilapia (Oreochromis mossambicus, Peters). Aquaculture 87: 145-154.

**Davies, S.J., Williamson, J., Robinson, M. and Bateson, R.I. 1989**. Practical inclusion levels of common animal by-products in complete diets for tilapia (Oreochromis mossambicus,Peters). In: Proc. 3rd Intl. Symp. on Feeding and Nutr. Fish. Tuba, Japan. pp. 325-332.

**Dror Y., Cohen Y., Yerushalmi-Rozen R., 2006**. Structure of gum arabic in aqueous solution. Journal of Polymer Science: Part B: Polymer Physics, 44, 3265–3271.

**El-Saidy, D. M. S. D. And Gaber, M. M. A., 1997**. Total replacement of fish meal by soybean meal, with various percentages of supplemental L-methionine, in diets for Nile tilapia Oreochromis niloticus fry. Ann. Agric. Sci.Moshtohor, 35: 1223–1238.

**El-Saidy, D. M. S. D. And Gaber, M. M. A., 2004**. Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish

**El-Saidy, D. M. S. D., Magdy, M. A. G. And Abdelshafy, A .S. A., 2005**. Evaluation of Cluster bean meal, Cyamposis tetragonoloba as a dietary protein source for common carp, Cyprinus carpio, L. J. World Aquacul. Soc., 36: 311–319.

**El-Sayed, A. F. M., 1999**. Alternative dietary protein source for farmed tilapia, Oreochromis spp. Aquaculture, 179: 149–168.

**El-Sayed, A. M., Mansour, C. R. and Ezzat, A. A. 2003**. Effects of dietary protein levels on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock reared at different water salinities. Aquaculture 220: 619-632.

**El-Sayed, A.-F.M. 1987**. Protein and energy requirements of Tilapia zillii. Ph.D. Thesis, Michigan State University, East Lansing, Michigan, USA. 147 pp.

**El-Sayed, A.-F.M. 1990**. Long-term evaluation of cotton seed meal as a protein source for Nile tilapia, Oreochromis niloticus. Aquaculture 84: 315-320.

**El-Sayed, A.-F.M. 1992**. Effects of substituting fish meal with Azolla pinnata in practical diets for fingerling and adult Nile tilapia Oreochromis niloticus L. Aquacult. Fish. Manag. 23: 167-173.

**El-Sayed, A.-F.M. 1998**. Total replacement of fishmeal with animal protein sources in Nile tilapia, Oreochromis niloticus (L.), feeds. Aquacult. Res. 29 (4): 275-280.

**El-Sayed, A.-F.M. 1999**. Alternative dietary protein sources for farmed tilapia, Oreochromis spp. Aquaculture 179: 149-168.

**El-Sayed, A.-F.M. and Teshima, S. 1992**. Protein and energy requirement of Nile tilapia, Oreochromis niloticus, fry. Aquaculture 103: 55-63.

**El-Sayed, A.-F.M., Moyano, F.J. and Martinez, I. 2000**. Assessment of the effect of plant inhibitors on digestive protease of Nile tilapia using in vitro assays. Aquaculture International 8: 403-415.

**El-Sayed, A.M and Teshima, S. 1991**. Tilapia nutrition in aquaculture. Reviews in Aquatic Sciences 5(3-4): 247-265.

**El-Sayed, A.M. 1989**. Evaluation of semipurified test diets for Tilapia zillii fingerlings. J. World Aquacult. Soc. 20(4): 240-244.

**Fagbenro, O.A. 1993**. Observations on macadamia presscake as supplemental feed for monosex Tilapia guineensis (Pisces: Cichlidae). J. Aquacult. Tropics 7: 91-94.

**Fagbenro, O.A. 1994**. Dried fermented fish silage in diets for Oreochromis niloticus. Isr. J. Aquacult.-Bamidgeh 46(3): 140-147.

**Fagbenro, O.A. and Jauncey, K. 1994**. Chemical and nutritional quality of dried fermented fish silage and their nutritive value for tilapia (Oreochromis niloticus). Animal Feed Sci. Technol. 45(2): 167-176.

**Fagbenro, O.A., Jauncey, K. and Haylor, G. 1994**. Nutritive value of diets containing dried

**FAO (Food and Agriculture Organization of the United Nations). 1983**. Fish feeds and feeding in developing countries. ADCP/Rep/83/18, FAO, Rome, Italy. 93 pp.

**FAO (Food and Agriculture Organization of the United Nations). 2004**. Fishstat Plus. Aquaculture production 1950-2002.

**FAO, 2011**. Aquaculture development. 5. Use of wild fish as feed in aquaculture. FAO Technical Guidelines for Responsible Fisheries. No. 5, Suppl. 5. FAO, Rome, p. 79. FAO/WHO: 1969 evaluations of some pesticide residues in food. FAO/PL: 1969.

**Fasakin, E.A., Balogun, A.M. and Fasuru, B.E. 1999**. Use of duckweed, Spirodela polyrrhiza L. Schleiden, as a protein feedstuff in practical diets for tilapia, Oreochromis niloticus L. Aquacult. Res. 30: 313-318.

**Goodrum, L. J., Patel, A., Leykam, J. F. and Kieliszewski, M. J., 2000**. Gum arabic glycoprotein contains glycomodules of both extensin and arabinogalactan-glycoproteins. Phytochemistry, 54, 99-106.

**Gunasekera, R.M., Shim, K. F. and Lam, T.J. 1996b**. Influence of protein content of broodstock diets on larval quality and performance in Nile tilapia, Oreochromis niloticus (L.). Aquaculture 146: 245-259.

**Gunasekera, R.M., Shim, K.F. and Lam, T.J. 1996a**. Effect of dietary protein level on spawning performance and amino acid composition of eggs of Nile tilapia, Oreochromis niloticus (L.). Aquaculture 146:121-134.

**Hanley, F. 1987**. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in tilapia, Oreochromis niloticus (L). Aquaculture 66: 163- 179.

**Hidalgo, M. C., Sanz, A., Gallego, M. G., Suraez, M. D. And Higera, M., 1993**. Feeding of European eel, Anguilla anguilla. 1. Influence of dietary carbohydrate level. Comp. Biochem. Physiol., 105: 165-169.

**Islam, A.M., Phillips, G.O., Sljivo, M.J. and Williams, P.A., 1997**. A review of recent developments on the regulatory, structural and functional aspects of gum arabic. Food Hydrocolloids, 11, 493–505.

**Jackson, A. J. and Capper. B. S. 1982**. Investigations into the requirements of tilapia Sarotherodon mossambicus, for dietary methionine, lysine and arginine in semi-synthetic diets. Aquaculture 20: 289-297.

**Jackson, A.J., Capper, B.S and Matty, A.J. 1982**. Evaluation of some plant proteins in complete diets for the tilapia, Sarotherodon mossambicus. Aquaculture 27: 97-109.

**Kumari, J. and Sahoo, P.K., 2006**. Non- specific immune response of healthy and immunocompromised Asian catfish (Clarias batrachus) to several immostimulants. Aquaculture, 255, 133141.

**Leng, R.A., Stanbolie, J.H. and Bell, R. 1995**. Duckweed - a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development 7(1). 11 p. (http://www.cipav.org.co/lrrd/lrrd7/1/3.htm).

**Lim, C. and Dominy, W.G. 1991**. Utilization of plant proteins by warmwater fish. In: D.M. Akiyama and R.K.H. Tan (eds.). Proc. Aquaculture Feed Processing and Nutrition Workshop. Thailand and Indonesia, 19-25 Sept. 1991. pp. 163-172.

**Lim, C., Webster, C. D. And Lee, C.-S. (Eds.), 2008**. Alternative protein sources in aquaculture diets. The Haworth Press, New York, NY, pp. 571.

**Mahboob, S. And Sheri, A.N., 1997**. Growth performance of major, common and some Chinese carps as influenced by pond fertilization and feed supplementation in composite culture system. J. Aqua.Trop., 12: 201-207.

**Mansour, C.R. 1998**. Nutrient requirements of red tilapia fingerlings. M. Sc. Thesis, Fac. Sci. Univ. Alex. Egypt. 121 pp.

**NRC (National Research Council). 1983**. Nutrient Requirements of Warmwater Fishes and Shellfishes. National Academy of Science, Washington, DC, USA.

**Oduro-Boateng, F., and Bart-Plange, A. 1988**. Pito brewery waste as an alternative protein source to fishmeal in feeds for Tilapia busumana. In: Pullin R.S.V., Bhukaswan, T., Tonguthai, K., Maclean J.L. (eds.). 2nd Intl. Symp. on Tilapia in Aquaculture, ICLARM Conf. Proc. No. 15, Manila, Philippines pp. 357-360.

**Phillips AO, Phillips GO., 2011**. Biofunctional behaviour and health benefits of a specific Gum Arabic. Food Hydrocolloids, 25(2):165–169.

**Randall, R.C., Phillips, G.O., and Williams, P.A. 1988**. The role of the proteinaceous component on the emulsifying properties of gum arabic. Food Hydrocolloids, 2, 131–140.

**Riche, M., Trottier, N.L., Ku, P.K. and Garling, D.L. 2001**. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (Oreochromis niloticus) fed phytase pretreated soybean meal diets. Fish Physiol. Biochem. 25: 181-194.

**Rodriguez-Serna, M., Olvera-Novoa, M.A. and Carmona-Osalde, C. 1996**. Nutritional value of animal by-product meal in practical diets for Nile tilapia Oreochromis niloticus (L.) fry. Aquacult. Res. 27: 67-73.

**Skillicorn, P, Spira, W. and Journey, W. 1993**. Duckweed aquaculture. a new aquatic farming system for developing countries. The World Bank, Washington, DC., USA. 76 pp.

**Tacon, A.G.J. 1993**. Feed ingredients for warmwater fish: fish meal and other processed feedstuffs. FAO Fisheries Circular No. 856, Rome. 64 pp.

**Tacon, A.G.J. and Jackson, A.J. 1985**. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey, C.B., Mackie, A.M. and Bell, J.G. (eds.). Nutrition and Feeding in Fish. Academic Press, London. pp. 119-145.

**Tacon, A.G.J., Jauncey, K., Falaye, A., Pantah, M., MacGowen , I. and Stafford, E. 1983**. The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for Oreochromis niloticus. In: Fishelson, J. and Yaron, Z. (eds.). Proc. 1st Intl. Symp. on Tilapia in Aquaculture. Tel Aviv Univ. Press, Israel. pp. 356-365.

**Teshima, S. and Kanazawa, A. 1988**. Nutritive value of methionine-enriched soy plastein for Oreochromis niloticus fry. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L. (eds.). 2nd Intl. Symp. on Tilapia in Aquaculture, ICLARM Conf. Proc. No. 15, Manila, Philippines. pp. 393-399.

**Viola, S. and Zohar, G. 1984**. Nutrition studies with market size hybrids of tilapia

**Viola, S., Arieli, Y., and Zohar, G. 1988**. Animal protein-free feeds for hybrid tilapia (Oreochromis niloticus x O. aureus) in intensive culture. Aquaculture 75: 115-125.

**Viola, S., Zohar, G. and Arieli, Y. 1986**. Phosphorus requirements and its availability from different sources for intensive pond culture species in Israel. Part 1. Tilapia. Bamidgeh 38 (1): 3-12.

**Watanabe, W.O., Clark, J.H., Dunham, J.B., Wicklund, R.I. and Olla, B.L. .1990**. Culture of Florida red tilapia in marine cages: the effects of stocking and dietary protein on growth. Aquaculture 90: 123-134.

**Wilson, R.P., Freeman, D.W. and Poe, E.W. 1984**. Three types of catfish offal meals for channel catfish fingerlings. Prog. Fish Cult. 46: 126-132.

**Winfree, R. A. and Stickney, R. R. 1981**. Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. J. Nutr. 111(6): 1001-1012.

**Wu, Y.V., Rosati, R.R., Sessa, D. and Brown, P.B. 1995**. Utilization of corn gluten feed by Nile tilapia. Prog. Fish Cult. 57: 305-309.