INTRODUCTION

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In a world where wild capture fisheries are becoming increasingly depleted, fish culture offers the possibility of commercial and home-grown protein sources. With the depletion of wild fish stocks by factors such as overfishing, pollution, habitat degradation and others, the pressures and incentives for commercial farming of food fishes increased dramatically.

In Egypt, fish production is still insufficient to fulfill the needs of the continuously increasing population, although there are vast aquatic resources including fresh, brackish and marine waters. Hence, proper management of the natural fishery and optimum utilization of different water sources are urgently needed to achieve higher fish production rates. In addition, aquaculture sector has a great potential and provides a more reliable source for local fish production.

Nowadays, fish culture has greatly improved not only in Egypt but allover the world, in order to improve the quality and quantity of different types of farmed fish and shrimp. On the other hand, Egypt faced a falling trend production in red meat quantities and subsequently much attention was given to fish production to compensate meat production.

Therefore, aquaculture is one of the best ways for exploitation the existing water bodies and constructed ponds (Hepher and Pruginin, 1981). The potential of local fish farming in freshwater ponds is so great due to the suitable climate and fertility of the lands (Hassanen, 1986). Ishak (1985) mentioned that the interest of aquaculture potential in Egypt has been directed mainly towards the development of pond culture either in shallow or deep ponds.

Tilapia species represented the third largest cosmopolitan group of farmed finfish species in 1994 after Cyprinids and Salmonids. Tilapia production within 72 different countries in 1994 accounted for 86.7% of the total world production as reported by FAO (1996). Moreover, the production of tilapia in developing countries (including Asian region) accounted for 97.8% (585, 897 million tons) of total production for the same year.

According to the informations given in the yearbook of fishery statistics FAO (1998), the global production in 1997 of tilapias fish catch from natural resources was 130, 992 million tons, while those from fish aquaculture was 30, 416 million tons (total 161, 408 million tons). On the other hand, the percentage global production of tilapias represented about 34.15% of the gross production of all fish catch from natural resources. While the percentage global production of tilapias represented 41.41% of all fish catch from fish aquaculture.

Feeds represent the major variable operating costs in fish farming, and optimizing their use represents an opportunity for many farmers to increase or maintain the profitability of their farms.

Generally, fish require diets relatively high in the protein content because of their poor utilization of carbohydrates as energy sources

(Wilson & Halver, 1986 and Degani *et al.*, 1989). Some observations on the effects of varying dietary protein level and every content on the growth, feed conversion and protein utilization of different tilapia species have been reported by Winfree & Stickney (1981), Jauncey (1982) and Siddiqui *et al.* (1988).

Formulation of fish diet with different levels of protein and energy depend mostly on fish meal, which is a rather expensive item for raising fish, especially for the developing countries including Egypt. The increasing demand on fish meal for aquacultural purposes has encouraged many workers to look for other sources of unconventional proteins that can replace fish meal, at least partially in fish diets. Many studies and efforts have been devoted concerning the dietary replacement of fish meal within compound aqua feeds for tilapia with alternative protein sources or fish meal replacers including, fishery and terrestrial animal by-product meals, oilseed meals and by-products, aquatic plant, single-cell protein, and legumes and cell by-products.

Preliminary feeding trials with fish indicated that fermented silage are nutritionally equivalent to fish meal (FAO, 1983). In view of the enhanced storage properties of these acid preservation techniques animal/ carbohydrates silage hold particular promise for use within the subtropical / tropical regions where conventional freezing techniques are expensive or not available (Jackson *et al.*, 1984 and Tacon & Jackson, 1985).

The present study evaluates co-dried fermented silage (FFS), boiled soybean meal (BSM) and mixture of dried fermented fish silage & treated soybean meal (1:1 w/w) ratio (MIX) as protein sources (partially replacing fish meal) in dry diets for three stages of *O. niloticus* fry, fingerlings and grow-out in order to :.

a) Determine the optimum inclusion level of fermented fish silage, boiled soybean meal and a mixture of both substances (FFS x BSM) (1:1 w/w) as fish meal replacers to give maximum growth performance and feed utilization.

b) Examine the effect of these test diets on the chemical composition (crude protein, total lipids, ash and water contents) and liver metabolites (protein, lipid and glycogen) of fish.

c) Evaluate the economy of the test diets.

Historical Review

Balarin and Haller (1982) determined the protein requirements of various size groups of tilapia and they concluded that 35-50% protein diet was needed for weight one gram; 30-40% for weight 1-5 gram; 25-30% for weight 5-25 gram and 20-25% for weight 25 gram or greater.

Viola and Arieli (1982) recorded a 20% increase in growth rate for tilapia fed 30% protein diet when compared with those fed 25% protein diet. Viola and Zohar (1984) recommended for tilapia culture a 25% protein feed in which the protein is derived partly from animal and plant sources. Zohar *et al.* (1984) used a 30% protein pelleted diet for tilapia (*O. niloticus*), and obtained a yield more than 50 tons/ha, with food conversion ratio of less than 1.0:2.5 compared to five different artificial diets contained 18, 22, 23, 29 or 32% crude protein.

growth rate and normalized biomass index were increased with the increasing the feeding level from 1% to 5%.

1. Fish Silage

A wide variety of fishery by-products have been evaluated and used in aqua feeds including; fish silage, fish protein hydrolysates and shrimp head meal, Krill meal and squid meal. The two techniques which have deals ensiling through chemical acidification (acid preserved silage) or bacterial fermentation (fermented silage) and protein hydrolysis under controlled conditions employing selected exogenous enzymes.

Espe *et al.* (1991) mentioned that, fish silage stored without stirring formed three layers containing protein, tyrosin but also cystine and tryptophan accumulated in the sediments, their amino acids were low in the clear aqueous layer. Acid fish silage is produced by the addition of external acids (both organic and inorganic acids). Propionic acid is often used not only for its antibacterial properties but also because it prevents mould growth. Mould growths in particular *Aspergillus flavus* have been showed in formic acid, silage stored in tropical condition even at pH less than 4.0 (Kompiang *et al.*, 1980).

Some preparing and feeding trials were studied by various investigators for acid fish silage to minimize an optimum percent of the acid which must be used to ensilage method (Gildberg & Raa, 1977 and Strom *et al.*, 1988). They reported that, 0.2% propionic acid ihibited *Aspergillus flavus* growth at pH 4.5. On the other hand, Austreng (1982) postulated that, the diets containing silage to which 0.5% propionic acid had been added were unpalatable to Atlantic salmon. Such observation was not present if formic acid was used. Rungruangsak and Utne (1981) reported that, the acids tested formic, hydrochloric and sulphuric greatly reduced dietary feed intake at high silage levels (97% silage).

Rungruangsak and Utene (1981) mentioned that, good results have generally been obtained with diets containing fish silage. Using diets containing 100% HCL silage produced growth in rainbow trout equivalent to that obtained with freshwaste fish (control diet). Similar results were also reported by Asgard & Austreng (1981) and Jackson *et al.* (1984) by using salmonids and Atlantic salmon co-dried sulphuric propionic fish silage as a replacement for fish meal in rainbow trout diet (Hardy *et al.*, 1984). This was in contrast with the normal uses of acid fish silage as liquid to which is added usually fish meal, together with oils, vitamins, minerals and binder before feeding as most feed pellets.

Stone *et al.* (1989) studied the apparent digestibility and utilization of fish protein subjected to the ensilaging process, comparing with fish meal in dry diets fed to rainbow tout (*Salmo gairdneri*). They reported that, digestibility values were higher for the acid fish silage than for fish meal, and the fish silage were not efficiently utilized for growth. Whole fish body or fish processing wastes were found to be equivalent sources of nitrogen provided the degree of autolyses and the diets were otherwise nutritionally balanced.

Silage production is also possible by lactic acid bacterial fermentation. To undergo proper fermentation the raw material must contain lactic acid bacteria, a suitable nutritional substrate for the bacteria and temperature compatible with rapid growth (Raa & Gildberg, 1982). Although lactic acid bacteria are invariably present in the raw material and a starter culture is not required (Kompiang *et al.*, 1980a & b), while the inoculation of material with fermented starter culture is recommended (James *et al.*, 1977). To favor, the growth of lactic acid bacteria as opposite to spoilage bacteria specific fermented substrate is mixed with the minced raw material to be ensiled.

FAO (1983) reported that, under favorable conditions the lactic acid product reduces the pH of silage, preserving it from spoilage and encouraging autolysis by naturally occurring protease enzymes. Preliminary feeding trials with fish indicated that fermented silage are nutritionally equivalent to fish meal.

Silage by anaerobic microbial fermentation (fermented silage) is preferred in developing countries because it is cheaper to produce, involves simple artisanal technology which is adaptable cottages level and possesses good strong properties (Dong *et al.*, 1993). As fish silage is liquid, it is bulky and difficult to transport, stir or store, hence it has to be dried mixed with other dry ingredients or filler material.

Molasses added at a ratio of 1:10 (w/w) molasses to fish are particularly effective (Kompiang *et al.*, 1980b and Raa & Gildberg, 1982). A good trial was prepared by Fagbenro *et al.* (1994) when using fermented silage from minced ungutted mixed sex tilapias (<100 gm) with 5% sugar beet molasses and 2% *Lactobacillus plantarum* (NCIMB 11974, NCIMB Ltd., Aberdeen Scotland), as a starter culture.

Several methods of removing or reducing the water content of fish silage's include sun drying, kiln drying, spray drying, vacuum evaporation, drum drying or co-drying (Jayawardena *et al.*, 1980). Co-dried silage have been evaluated as protein feed stuff in diets fed to tilapias fish (Djajasewaka & Djajadiredja, 1980 and Phromkunthong & Chetanon, 1987).

However, information on the evaluation of co-dried fermented silage in fish diets is limited and has not been directly compared to fish meal in fish feeding trials (Lopez, 1990). Further studies on the preparation, preservation and properties of fermented tilapia silage were conducted by (Fagbenro & Jauncey, 1993a, b and 1994). Their studies evaluated co-dried fermented tilapia silage, treated soybean meal and mixture of co-dried fermented silage and treated soybean meal (1:1) every one replacer used separately as protein source (partially replacing fish meal) in dry diets for different ages of tilapia (*O. niloticus*). The effects of these food replacers on growth performance, feed conversion, protein utilization, digestibility and carcass composition of experimented fish were investigated.

Fagbenro and Jauncey (1993a & b) found that, lactic acid fermented fish silage (FFS) could be stored at 30C° for six months with little or no nitrogen loss and change in nutritional quality, and reported that protein autolysis was directly related to ambient temperature. Moreover, experimental aqua feeds containing FFS were reported to have very good water stability and low nitrogen loss irrespective of the binder used (Fagbenro and Jauncey, 1995).

2. Soybean meal (SBM) as fish meal replacer :

Soybean meal is generally considered to be one of the best readily available plant protein sources in terms of its protein quality and essential amino acids (EAA) profile (with the exception of methionin) like most other plant proteins. However, it contains a wide variety of endogenous anti-nutrients which require removal or inactivation through processing prior to usage within aqua feeds (Tacon, 1995). Despite this, numerous studies have been conducted using processed soybean as a fish meal replacer with tilapia feeds. Successful trials were conducted for replacing prepressed solvent extract as for full fat soybean meal with or without methionin supplementation's up to 75% of FM within diets fed to *O. niloticus* fry (Pantha, 1982 and Tacon *et al.*, 1983), *O. mossambicus* (Jackson *et al.*, 1982) and tilapia hybrids (Shiau *et al.*, 1989). The dietary supplementation of SBM based diets with limiting EAA diet did not improve the nutritional value of SBM for tilapia since they appear to gain little or no benefit from crystalline EAA (Viola & Arieli, 1983a & b and Teshima & Kanazawa, 1988). Similarly, Viola *et al.*, (1988) studied the limiting factors in SBM for tilapia hybrids (*O. niloticus* x *O. aureus)* reared within outdoor ponds and noticed that, fish fed a SBM-based diet supplemented with lys., met., lipid and di-calcium phosphate (DCP) had the same performance as fish fed FM-based diet (at 100% substitution level) without negative effects on fish growth. The authors reported that phosphorus (and not deficient EAA) was the limiting factor in SBM under the prevailing experimental conditions. Similar studies was also made by Viola *et al.* (1986a, b); Viola *et al.* (1994) and Viola *et al.* (1994a) and found that dietary supplementation with crystalline lys. to

SBM based diets was ineffective at 25 – 35% dietary crude protein levels, reducing lys/ protein levels impaired fish growth.

The apparent non essentiality of supplemental crystalline EAA for tilapia has also been reported for *T. zillii* fed as same meal based diet (El-Sayed, 1987) and for *O. niloticus* fed a cotton seed meal based diet (El-Sayed, 1990).

Pantha (1982) found that no difference in the growth performance and food utilization of *O. niloticus* fry when fed a diet within 40% protein level in diet which supplied by herring meal and where 75% of the herring meal was replaced by full fat soybean and supplemented with d, L methionine.

NRC (1983) reported that, the recommended maximum levels for feed ingredients fish fry starter diets are as follows, fish meal (no limit), poultry by-product meal (35%), cotton seed meal (15%), soybean meal (20%), corn gluten meal (10%), brewers yeast (30%), ground wheat (20%) and tallow (12%).

Viola and Arieli (1983a) reported that, the soybean meal can be used to replace up to half of the fish meal in tilapia feeds of 25% protein without requiring any other supplements.

It must be noted that, the using of SBM as a partial replacer in tilapia feeds is affecting by the dietary protein level. Davis and Stickney (1978) fed *O. aureus* with SBM-based diets at dietary protein levels ranging from 15 to 36% and found that, negative result for fish growth at 15% crude protein level while SBM could totally replace FM within diets containing 36% crude protein. Shiau *et al.* (1987) stated results with *O. niloticus* x *O. aureus* hybrids and reported that, FM could be partially replaced by SBM within diets containing sub-optimal protein levels (24%), whereas at optimum protein levels (32%) the dietary replacement of FM with 30% SBM significantly depressed fish performance. Wee and Shu (1989) reported that the nutritional value of full-fat SBM for *O. niloticus* was improved and the trypsin inhibitor activity level reduced by boiling for one hour prior to usage. Their results must be considered an extended studies for the trials of Wassef *et al.* (1988), when they suggested that proteolytic activities of papain and trypsin on soya meal were increased after the meals were deffatted or prepared from germinated soya beans or digested for long periods. Moreover, they reported that seedling growth repressed the inhibitors of the protease activities in the cotyledons and induced the development of high levels of alpha amino nitrogen.

Wolf (1983) reported that, digestion of protein from leguminous seeds can be limited by endogenous protein inhibitors. Also, Rackis (1966) and Liener (1981) stated that in soybeans these inhibitors are described in terms of their potential to inhibit trypsin and are referred to as trypsin inhibitors. The use of soybean meal as a replacement for more expensive fish meal has been investigated by Tacon and Jackson (1985). However, the results of such studies suggest that further improvements in feeding value of soybean fish feed is required because digestion of soybean protein is inefficient (Everson *et al.*, 1943; Bates, *et al.*, 1977 and Tacon & Jackson, 1985).

Some attentions has also been given to the use of other oil seed meals and by products as source of dietary protein for tilapia including, groundnut, sunflower, rape seeds, sesame seeds, copra, macadamia, and palm kernel (Jackson *et al.* 1982; Fagbenro, 1988; El-Sayed, 1987; Fagbenro, 1993; Omoregie & Ogbemudia, 1993 and Balogun & Fagbenre, 1995).

Cotton seed meal and cake (CSC) has also been used both as a feed and fertilizer within semi-intensive pond farming systems. For example CSC (42% crude protein) was used as the sole feed input for *O. niloticus* reared within earthen ponds fertilized with cattle manure, fish being fed cotton seed cake at 3 and 6% of body weight/ day over a 100 day feeding period grew from 88 gm to 303 gm and 321 gm (Middendorp, 1995a, b and Middendorp & Huisman, 1995). Middendorp (1995) stated that there is no beneficial effect of mixing brewery waste with CSC as feed inputs, with fish performance tending to be negatively affected by the dietary addition of brewery waste.

Cereal by-product such as maize gluten meal, gluten feed, grain distillers and brewery waste has also been successfully used as dietary protein sources for tilapia. For example, the growth of *O. niloticus* fingerlings fed maize distillers grains with solubles, gluten meal and gluten feed as partial protein sources was better than that of fish fed a FM-based content diet (Wu *et al.*, 1994, 1995 and Tudor *et al.*, 1995). Similarly, pito brewery waste has been used as a full dietary replacement for FM for tilapia species (Pouomogne, 1995).

3. A Mixture of both FFS and SBM

Hastings (1973) established modern techniques of fish feed technology including ration formulation, stocking of feed elements, grinding, mixing and pelleting. His feeding trials were conducted on *Tilapia nilotica* and *Clarias lazera*, using two different 30% of protein rations, one with only vegetable protein sources and the other with one quarter of the protein from animal sources. Results of two trials showed that greater tilapia production was obtained from the vegetable protein diet (5150kg/ha/year) these studies showed that the feed conversion was excellent (1.17:1.00) and the growth of fish was (0.85 gm/fish /day).

Davis and Stickney (1978) reported an optimum protein requirement level of 36% for tilapia (*Tilapia aurea*). This value was reduced to 29% when using a mixture of fish meal and soybean meal (1:1) as the major protein source.

While Lapie and Bigueras-Benitez (1992) found no difference in the growth and feed efficiency between Nile tilapia (*O. niloticus*) fed a formic acid preserved fish silage (FS) blended with FM (1:1 ratio) or FM-based ration, although growth performance was reduced when the FS:FM ration was increased to 3:1.

Fagbenro (1994) reported also that no difference in the performance between *O. niloticus* fed a 30% crude protein ration containing blended FFS (1:1 w/w) with soybean meal, poultry by-product meal, hydrolysed feather meal or meat and bone meal with fish fed a control FM-based rations.

Fagbenro *et al.* (1994) found that up to 75% of FM protein could be successfully replaced with a dried 1:1 mixture of blended FFS: soybean meal in feeds for all male *O. niloticus*.

Fagbenro *et al.* (1994) blended soybean meal with the wet tilapia silage (1;1, w/w), then four dry semi-purified diets were formulated separately for *O. niloticus* (30% protein) and *Clarias gariepinus* (40% protein) and contained increased levels of the co-dried FS:SBM as replacement for fish meal. The previous authors found that, diets containing co-dried FS:SBM supported weight gains and growth rates similar to those in the control treatment without significant differences ($p>0.05$). They also stated that, feed conversion, protein efficiency ratio and protein productive value (protein retention) followed a similar trend with growth performance values. Apparent protein digestibility decreased in both fish species with increasing dietary level co-dried FS:SBM.

Other sources of diets :

Tiews *et al.* (1979) made a greatest research effort centered on the yeast, single cell protein and particular alkaline/ petrochemical yeast (*Candida lipolytica*) i.e. Toprina and obtained equivalent growth to fish meal based rations.

Relatively few studies have been reported on the direct use of dried algae. Single cell protein has been found to have a lower feed value for fish than higher yeast, bacterial single cell protein or fish meal (*Spirulina maxima*) (Matty & Smith, 1978; Atack *et al.*, 1979 and Atack & Matty, 1979). Appler and Jauncey (1983) with *O. niloticus* indicated that, certain dried algae meals (*Cladophora glomerte, Scenedesmus obliques, Chlorella spp., Euglena spp.*) may offer particular promise as a partial dietary replacement for fish meal within rations at relatively low dietary inclusion levels (20% algae, SCP).

Relatively few studies have been conducted concerning the use of aquatic plants as feed ingredients with tilapia feeds, from them Almazan *et al.* (1986), Edwards (1987), Okeyo (1988), Chiayvareesajja *et al.* (1990), Journey *et al.* (1990), Klinnavee *et al.* (1990), Mbagwu *et al.* (1990), Wee (1991) and El-Sayed (1992).

Tacon and Jackson (1985) studied the influence of terrestrial by-product meals which included; poultry by-product meal, hydrolyzed feathermeal, blood meal and meat and bone meal when using as fish meal replacer. They reported that despite their usually high crude protein content, these fish meal replacers are usually deficient in one or more essential amino acids.

Some long term feeding trials conducted by Otubusin (1987) with cage reared *O. niloticus* fingerlings at a period of 120 days (3 gm initial body weight) concerning the use of blood meal as a FM replacer, found that dietary BM inclusion levels above 50% of FM protein significantly reduced fish performance (10% replacement level being the most efficient).

Davies *et al.* (1989) found that, optimum meat blood meal MBM/blood meal BM ratios, could effectively replace up to 75% of the fish meal in diets fed to Mozambique tilapia (*O. mossambicus*) fry over a seven week period. Zohar *et al.* (1984) and Davies *et al.* (1989) reported a poor growth in some tilapia species (*O. niloticus, O. mossambicus* and tilapia hybrid) when fed hydrolyzed feather meal (HFM) or HFM-based diets. Falaye (1982) and Bishop *et al.* (1995) reported that, HFM could replace up to 50% and 66% of the FM or FM : MBM within diets for *O. niloticus* fingerlings and fry with no loss in growth performance.

Also, the results reported concerning the use of leguminous and cereal plants and their by-products as FM- replacers have been promosing. However, like the majority of most plant proteins, they may contain a wide variety of anti-nutrients, which could require removal or inactivation through processing prior to usage within aqua feeds (Tacon, 1995).

A number of studies have been conducted by Jackson *et al.* (1982), Wee and Wang (1987), Santiago *et al.* (1988), Chikafumbwa *et al.* (1991) and Osman *et al.* (1996) on using of leucaena leaf meal in tilapia feeds, with similar results. De Silva & Gunasekera (1989) and Ng & Wee (1989) have also reported similar results on feeding of *O. niloticus* on legumes, including cassava leaf meal and green gram.