PROTEIN NUTRITION OF FARMED TILAPIA: SEARCHING FOR UNCONVENTIONAL SOURCES

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Abstract

Tilapia are the third most important cultured fish group in the world, after carps and Salmonids. Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4% during 1970–2002. They are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of total farmed finfish in 2002. Feeding represents over 50% of the operational costs of aquaculture. Fish meal has been widely used as a main conventional protein source in aquafeeds. However, the dependence on fish meal is expected to decline due to the shortage in world production of fish meal, and increased demand for fish meal in feeds for livestock and poultry industries. Therefore, partial or total replacement of fish meal with less expensive, locally available protein sources will become inevitable. Many attempts have been conducted alongside this line. The present review throws the light on alternative dietary protein sources for farmed tilapia, with emphasis on the most cost effective, commonly used sources, such as fishery by-products, terrestrial animal by-products, oilseed plants, aquatic plants, single cell proteins, grain legumes, plant protein concentrates and cereal by-products. The nutritive values, inclusion levels, constraints and economic evaluation of these sources are discussed.

Introduction

Tilapia are the third most important cultured fish group in the world, after carps and salmonids. Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4% during 1970–2002. They are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of total farmed finfish in 2002 (FAO, 2004).

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

Protein requirements

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: (1) were conducted indoor, (2) were short-term, (3) may not be directly applied in field trials, and (4) 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). Therefore, it is no surprise that the results of protein requirements of tilapia are varying and sometimes contradictory, as shown in Table 1.

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 (Table 1) (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 require 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*., 1996a,b; Siddiqui *et al*., 1998; El-Sayed *et al*., 2003).

Amino acid requirements

Despite that tilapia require the 10 essential amino acids (arginine, lysine, histidine, threonine, valine, leucine, isoleucine, methionine, phenylalanine, and tryptophan), specific EAA requirements of most farmed tilapias have not been determined. Few studies have considered EAA requirements of Nile tilapia, *O. niloticus*, and *0. mossambicus*. These requirements are summarized in Table 2.

Table 1. Protein requirements of cultured tilapia.

Species and life stage	Weight (g)	Protein source	Requirement	References
O. niloticus				
Fry	0.012	FM[1]	45%	El-Sayed & Teshima, 1992
	0.51	FM	40	Al Hafedh, 1999
	0.80	FM	40	Siddiqui *et al*., 1988
Fingerlings	2.4	Casein/Gelatin	35	Abdelghany, 2000a
	3.50	Casein	30	Wang *et al*., 1985
	6.1-16.5	FM	30	De Silva & Radampola, 1990
	45-264	FM	30	Al Hafedh, 1999
Broodstock		FM/SBM	40	El-Sayed *et al*., 2003
		FM	45	Siddiqui *et al*., 1998
		Casein/Gelatin	35-40	Gunasekera *et al*., 1996a,b
O. mossambicus	Fry	FM	40-50	Jauncey, 1982; Jauncey and Ross, 1982
	6-30	FM	30-35	Jauncey & Ross, 1982
O. aureus	0.30-0.50	SBM or FM	36	Davis & Stickney, 1978
	2.50	Casein/albumen	56	Winfree & Stickney, 1981
	7.5	Casein/albumen	34	Winfree & Stickney, 1981
T. zillii	1.35-1.80	Casein	35	Mazid *et al*., 1979
	1.4-1.7	Casein/Gelatin	35-40	El-Sayed, 1987; Teshima *et al*. 1978
O. niloticus X *O. aureus*	145-242	FM+CSM	20	Cisse, 1988
	0.6-1.1	FM	32	Shiau and Peng, 1993
	21	SBM	28	Twibell and Brown, 1998
O. niloticus X *O. hornorum*	1.24	-	32	Luquet, 1989
O. mossambicus X O. hornorum	8.87	-	28	Watanabe *et al*., 1990

Note: FM - Fish Meal, SBM - Soybean Meal, CSM - Cotton Seed Meal

The EAA requirements of tilapia may depend on the species and amino acid source (e.g. crystalline AA vs. casein/gelatin). For example, the performance of tilapia fed crystalline EAA-based diets was lower than those fed casein or casein/gelatin diets (Jackson and Capper, 1982; Teshima *et al*., 1985), presumably due to the low pH of the AA-based diets. More work is needed in this area, since other studies indicated that lowering food pH enhanced the appetite of *Tilapia zillii* for food (Adams *et al*., 1988).

Tilapia requirements for sulfur–containing AA can be met by methionine or a methionine/cystine mixture. Recent studies recommended a methionine:cystine ratio of 50:50 for the best performance of Nile tilapia (Abdelghany, 2000b). Similarly, the requirement of tilapia for phenylalanine (aromatic amino acid) could partially be met by tyrosine (NRC, 1993).

Table 2. Essential amino acid requirements of *O. mossambicus* and *O. niloticus* as % of dietary protein and of total diet (in parenthesis).

Amino Acid	Requirement			
	O. mossamicus[1]	*O. mossambicus*[2]	*O. niloticus*[3]	*O. niloticus*[4]
Lysine	4.05 (1.62)	3.78 (1.51)	5.12 (1.43)	---
Arginine	3.80 (1.52)	2.82 (1.13)	4.20 (1.18)	4.1
Histidine		1.05 (0.42)	1.72 (0.48)	1.5
Threonine		2.93 (1.17)	3.75 (1.05)	3.3
Valine		2.20 (0.88)	2.80 (0.78)	3.0
Leucine		3.40 (1.35)	3.39 (0.95)	4.3
Isoleucine		2.01 (0.80)	3.11 (0.87)	2.6
Methionine	1.33 (0.53)	0.99 (0.40)	2.68 (0.75)	1.3
Cystine			0.53	2.1
Phenylalanine		2.50 (1.00)	3.75 (1.05)	3.2
Tyrosine			1.79	1.6
Tryptophan		0.43 (0.17)	1.00 (0.28)	0.6

[1]Jackson and Capper (1982); [2]Jauncey *et al*. (1983), [3]Santiago and Lovell (1988); [4]Fagbenro (2000).

Protein sources

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 sought. 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 aquafeed 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 aquafeeds. 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. 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).

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 up to 15% and 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. High inclusion levels of COS should be tested in order to determine the proper inclusion level.

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 antinutrients, 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 mt/ha/year (Leng *et al*., 1995). Duckweed can be used as a single food source for farmed tilapia (Fasakin *et al*., 1999). 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 mt/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 *Potamogeton gramineous* 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 antinutrients 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 use 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 (Table 3). 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

Amino acid versus mineral supplementation?

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.

Table 3. Digestibility by tilapia of some dietary protein sources.

Source	1[a]	2[b]	3[a]	4[a]	5[a]	6[a]	7[c]	8[a]
Fish meal	92.2	85	72	86	92	94		
Fish silage								
Meat and bone meal	92.2	68-78						
Poultry offal meal				74				
Shrimp meal					87		74	
Silkworm pupa meal	91.1							
Brewers grains			62	63			63	
Azolla					75			
Casein					97.2			
Copra meal					56	81		
Corn gluten meal	90.7				97			
Cottonseed meal			31				90	
Cottonseed cake						90		
Groundnut meal			79					
Soybean meal	90.9	94		91	93	93	91	94.4
Full-fat toasted soybean								90
Sunflower seed meal								
Sunflower seed cake								
Pea seed meal								92

1 Watanabe *et al*. (1996), 2 Popma (1982), 3 Luquet (1989), 4 Hanley (1987), 5 Lorico-Querijero and Chiu (1989), 6 Moreau (1996), 7 Degani *et al*. (1997), and 8 Fontainhas-Fernandes *et al*. (1999); a = Nile tilapia, b = *O. aureus* and c = *O. aureus* x *O. niloticus*.

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 antinutritional 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.

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