ALTERNATIVE PROTEIN SOURCES IN AQUACULTURE DIETS: A REVIEW

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ABSTRACT

Fish meal has been widely used as the main conventional protein source in aqua feeds. 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 alternative protein sources will become inevitable. Many attempts have been conducted alongside this line. The present review throws light on alternative dietary protein sources for aqua feeds, 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 different processing methods involved in removing the ant nutritional compounds and improve the protein content in the plant protein sources.

Keywords: Alternative, Aqua feeds, Ant nutritional compounds.

Introduction

Aquaculture shares the same challenge with agriculture to increase food supply and this brings about competition in the use of feeds (for livestock and fish farming) and fertilizers (for farm crops). Since 1990, there have been recurrent shortages of major fish feedstuffs, particularly rice bran, yellow corn, soybean meal, and fishmeal (Sogbesan, 2014), and the supply has not increased. With the poultry and livestock industries projected to expand at 6-8% annually (Sogbesan, 2014), the aquaculture industry is finding it increasingly more difficult to source for critical feed ingredients.

The future growth of aquaculture industry depends upon the availability of suitable and economical feeds. Information on the type, quality, quantity, seasonality and cost of fish feeds is essential in determining the appropriate production strategy. For aquaculture to supply the populations projected growing demand of 1.74million tonnes deficit (Federal Department of Fisheries 2003), information should be available, especially on low-cost feeds that are less competitive. These should have replaceable capacity for fishmeal with the aim of making fish attain table size at reduced culture time and minimum production cost (Sogbesan 2014)

Hike in the price of fishmeal, and consequently compounded fish feed, has led to investigations into other alternative cheap sources of fish feed ingredients that will provide the required nutrient for fish at cheaper cost of production, since the cost of feeding is a major factor affecting the development of aquaculture enterprise in Nigeria (Sogbesan 2014).

In 1989, the Presidential Task Force on Alternative Formulation of Feed identified feeding of fish as one of the major problems facing the development of the aquaculture industry in Nigeria, and proffered possible solutions geared towards increasing fish production from this sector. One of the suggestions made was the utilization of non-convectional protein supplements of both animal and plant origin in practical fish diets. Webster *et al.* (2000) noted that, it is imperative for such practical fatty acids, Vitamins and Mineral salts needed by the fish. When these nutrients requirements are not met, the growth and health of fish are impaired; which ultimately lead to reduction in the fish farms output. Hence, the production of cost effective nutritionally and biological evaluation. The Non-Conventional feed Resources (NCFRs) are feedstuffs, that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production (Sogbesan 2014). The NCFRs are credited for the following reasons:

1. They are non-competitive in terms of human consumption.

2. They are very cheap by-products or wastes from agriculture, farm-made feeds and processing industries.

3. Their use is able to serve as a form of waste management and enhancing good sanitation.

The animal NCFRs are preferable to the plant NCFRs because of the fact that they don't contain anti-nutritional and anti-growth factors which are from the phytochemicals, and these factors do limit the inclusion of plant products in fish feed despite the available processing methods (Sogbesan 2014)

Nutrient Requirements

The nutrient balance of feed influence feed utilization and growth of fish. It is very essential to know the nutritional requirements particularly for protein, lipid and energy for optimum growth of a fish species as well as in formulating a balanced diet. For a nutrient to be essential to fish, it must have been demonstrated that its absence in a diet fed to fish will reduce growth, impair immune function and decrease reproductive capacity, cause observable clinical and sub-clinical disability or death (Luckey 1976 and Sogbesan *et al*. 2006a) According to Lovell (1989), dietary protein and energy levels are known to affect the growth and body composition of fish species.

Some authors (Phillips 1972; Prather and Lovell 1973; Shyong *et al*., 1998) observed that improper protein, energy and other nutrient levels in feed increased fish production cost especially the recurrent expenditure and deteriorates water quality. While insufficient energy in diets caused protein waste due to the increase proportion of dietary protein used for energy and the produced ammonia can pollute the water and make it unfit for fish culture. However, Daniels and Robinsons (1986) and Van der Meer *et al*. (1997) reported that excessive energy in diets could lead to increased body lipid deposition and growth reduction because of lack of necessary nutrient for growth. From the economic stand point, feed cost appears to be one of the major constraints against the expansion of aquaculture (Ayuba *et al.,* 2013). In fish feed formulation, protein and energy requirements of the species under culture is highly considered above all other nutrients (Abu *et al*., 2009).

Protein Requirements

Edwin (2009) reported that because protein is the most expensive part of fish feed, it is important to accurately determine the protein requirements for each species and size of cultured fish. Proteins are formed by linkages of individual amino acids. Although over 200 amino acids occur in nature, only about 20 amino acids are common. Of these, 10 are essential (indispensable) amino acids that cannot be synthesized by fish. The 10 essential amino acids that must be supplied by the diet are: methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Of these, lysine and methionine are often the first limiting amino acids. Fish feeds prepared with plant (soybean meal) protein typically are low in methionine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health. It is important to know and match the protein requirements and the amino acid requirements of each fish species reared.

Protein levels in aquaculture feeds generally average 18-20% for marine shrimp, 28-32% for catfish, 32-38% for tilapia, 38-42% for hybrid striped bass. Protein requirements usually are lower for herbivorous fish (plant eating) and omnivorous fish (plant-animal eaters) than they are for carnivorous (flesh-eating) fish, and are higher for fish reared in high density (recirculating aquaculture) than low density (pond aquaculture) systems (Edwin 2009). Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. Protein is used for fish growth if adequate levels of fats and carbohydrates are present in the diet. If not, protein may be used for energy and life support rather than growth.

The optimum percentage of protein in fish diets is influenced by several factors such as the following:

(i) Size of fish: Fish, like land animals, have higher protein requirements during early life than during later phases of growth.

(ii) Physiological function: Less protein is needed in a maintenance diet than in one fed for a rapid growth rate.

(iii) Protein quality: A protein that is deficient in one or more of the ten essential amino acids will produce less growth than a protein that is balanced in the essential amino acids or, more of a low quality protein is needed in the diet for maximum growth than a high quality protein.

(iv) Non-protein energy in the diet: If the diet is deficient in energy, the fish will use part of the protein to meet energy needs thus reducing the amount of dietary protein available for growth.

(v) Feeding rate: Fish fed to less than satiation, as frequently occurs in intensive pond culture of food fish, will benefit from diets containing higher percentages of protein than fish diets fed at or near the satiation rate.

(vi) Natural foods: If natural aquatic organisms contribute significantly to the daily food intake of the fish, the protein level in the prepared diet may be reduced. For example, aquatic fauna that are consumed by various fishes contain from 60 to 80 percent protein, thus, if in abundance, the supplementary diet would need only a very low percentage of protein.

(vii) Economics: The cost and availability of protein sources is a major factor in determining how much protein to use in commercial diets (FAO 2004)

Ferouz et al., (2012) states that fish diets typically contain between 20 and 55% crude protein, depending on the fish species. High quantities of fish meal are commonly used in these feeds to supply fish with essential proteins, amino acids and fatty acids. Channel catfish, Ictalurus punctatus, in contrast, are fed feeds containing 28-32% crude protein, most of which is supplied by soybean meal (Robinson and Li, 2002). Members of the carp family are fed feeds with protein contents varying from 0 to 35%, depending on species, where they are farmed, and life-history stage (Shivananda Murthy, 2002; Takeuchi et al., 2002). Fry and fingerling carp are fed feeds containing higher protein levels than are post-juvenile fish. Carp feeds intended for use in high-input culture systems contain 15-25% fish meal, and although this is a relatively low fish meal inclusion level, the tremendous increase in high-input carp culture has dramatically increased the amount of fish meal used by this production sector to about 17% of the total amount of fish meal used in all aqua feeds in 2000 (Barlow, 2000). The percentage of fish meal in feeds ranges from 55% for marine flatfish (flounder, turbot, and halibut) to 3% for catfish (channel catfish, African catfish). Carp average 5%, but this figure includes both high-input and low-input systems. Carp farming is converting to high-input systems, and this will increase the total use of fish meal in this production sector, despite an anticipated reduction in the percentage of fish meal used in feeds (Barlow, 2000). Carp feed production is anticipated to increase from about 7,000,000 mt in 2000 to 27,000,000 mt by 2010. Soybean meal will likely supply the bulk of protein in carp feeds of the future, but fish meal will continue to be used in feeds for fry and fingerling carp.

Amino Acid Requirements

For nutritional purposes, amino acids may be divided into two groups; the essential amino acids (EAA), and the non-essential amino acids (NEAA). EAA are those amino acids that cannot be synthesized within the animal body or at a rate sufficient to meet the physiological needs of the growing animal, and must therefore be supplied in a ready-made form in the diet. NEAA are those amino acids that can be synthesized in the body from a suitable carbon source and amino groups from other amino acids or simple compounds such as diammonium citrate, and consequently do not have to be supplied in a ready-made form in the diet.

The dietary EAA for fish and shrimp are as follows:

Threonine	Valine
Leucine	Isoleucine
Methionine	Tryptophan
Lysine	Histidine
Arginine	Phenylalanine

Although the NEAA are not dietary essential nutrients, they perform many essential functions at the cellular or metabolic level. They are termed dietary non-essential nutrients only because the body tissues can synthesize them on demand. In fact it is often quoted that the NEAA are physiologically so essential that the body ensures an adequate supply by synthesis. From a feed formulation viewpoint, it is important to know that the NEAA's cystine and tyrosine can be synthesized within the body from the EAA's methionine and phenylalanine respectively, and consequently the dietary requirement for these EAA is dependent on the concentration of the corresponding NEAA within the diet

Alternative Protein Sources

Many works have been done on the nutritional value of many alternative feed ingredients to supplement the dietary fishmeal-based diets. Several researches have shown that alternative protein sources derived from grains such as corn, wheat, barley, soy bean, cotton seed, fishmeal, and animal rendering products such as mechanically deboned meal, poultry by-product meal, meat and bone meal, maggot, Bambara groundnut, etc. can be used to formulate nutritious aquaculture feed at various levels of inclusions in different feeds.

If alternative protein sources were equal or superior in nutritional and economic value to fish meal, they would already be widely used in aqua feeds. All common alternative protein sources possess characteristics that make them inferior to fish meal. Some alternative protein sources

have inferior amino acid profiles to fish meal, while others contain constituents that lower nutritional value or lack constituents that are required to support normal fish growth. Research efforts are underway to identify these constituents, and, in the case of negative ones, develop ways of removing, inactivating or overcoming them. In the case of nutrients or constituents in fish meal that are missing in plant proteins, these must be identified, their optimum dietary level identified, and they must then be supplemented into plant protein-based aquafeeds (Ronald. W. Hardy. 2006.). Despite the negatives associated with alternative protein sources, they have always been used in aqua feeds to complement fish meal protein or lower feed cost. Given the current high cost of fish meal, there is intense pressure to re-evaluate common alternative protein sources to determine how best to use them in low-fish meal aqua feeds. Now and in the future, alternative proteins must be considered primary protein sources in aqua feeds, with fish meal used sparingly to complement the alternative proteins. Alternative Proteins fall into three general categories:

(1) Animal proteins from rendering or slaughter;

(2) Plant protein concentrates

(3) Novel proteins Such as single cell proteins, insect meals, especially products produced from seafood processing waste, and especially products derived from ethanol production. Animal proteins, such as poultry by-product meal, meat and bone meal, blood meal, and feather meal, have long been used in aqua feeds. In general, they are inferior to fish meal due to lower protein (amino acid) digestibilities, high ash levels in the case of poultry and meat and bone meals, high variability in quality, and, in the case of blood meal and feather meal, amino acid profiles that do not match the essential amino acid requirements of fish. However, they are less expensive protein sources than fish meal and, in general, palatable and free of anti-nutritional factors. As mentioned above, aqua feed manufactures have used these ingredients for decades at low levels in aqua feeds to lower costs. Now, the focus is on using higher percentages in aquafeeds for certain species of farmed fish and shrimp. When high quality animal and poultry by-product meals are tested, research shows that higher use levels are possible. Over the past several years, many studies have been published that document their nutritional value and optimum use levels in aqua feeds for a range of farmed fish (Bureau et al., 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 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 368 nutritionists with information on their advantages and disadvantages of such feed ingredients as well as their proper inclusion levels in tilapia feeds.(Udoet al., 2011) reported that diets with 20% of fishmeal replaced by meat-and-bone meals produced growth equivalent to the control diet in Gibel carp, (Udoet al., 2011) also reported that up to 47.6% of the fishmeal could be replaced with soybean meal in the diets of yellow perch (Perca flavescens) without affecting the feed consumption, weight gain, feed efficiency and survival of the fish.

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% 369 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.

 Poultry-by-Product

Nibea miichthioides and Carassius auratus gibelio grew well on diets with 50% of the fishmeal replaced by poultry- by- product meal (Udo, et al., 2011).

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, phytohaemaglutinin 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 etal., 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 200oC for 10min) 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 370 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 Potamogetongramineous 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 Elodeatrifoliate and Muyriophyllum spicatum have been reported to reduce tilapia performance. Ayuub Ayodele Ayoola(2010) observed that replacement of fishmeal with casein and soy flour meals in rainbow trout feed had a negative effect on growth performance. Plant protein tends to lower feed intake by reducing diet palatability when replacement levels are high, or by affecting the health of the fish in other ways, such as the condition describe as distal enteritis in Atlantic salmon and rainbow trout fed with high soybean meal (Refstie etal., 2000). Furthermore, some plant proteins contain phosphorus as phosphorus phytate. The phytate makes phosphorus in seeds unavailable to monogastric animals, including fish. Also, phytate interferes with the bioavailability of divalent trace elements, especially zinc, making it necessary to over fortify feeds to ensure adequate dietary zinc intake in fish feed containing 16 high level of phytate especially in the presence of high dietary calcium levels (Ayuub Ayodele Ayoola., 2010).

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.

Oso, et al., 2013 showed that Clarias gariepinus fingerlings fed up to 75% inclusion of Bambara groundnut in fish feed was satisfactorily acceptable without affecting growth and feed utilization.

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.

Amino Acid Scores for the Protein Feedstuffs

Reigh, (2008) reported that Most alternative plant protein supplements possess poorer amino acid profiles than the ingredients they replace (i.e., fish meal and soybean meal).replacement scores (RS) are based on a comparison of the amino acid composition and protein content of each ingredient in relation to that of fish meal or soybean meal, as follows:

$RS = (\sum EAA_{alt} / \sum EAA_{fish/soy}) \times (CP_{alt} / CP_{fish/soy})$

Where

$\sum EAA_{alt}$ = sum (%) of essential amino acids (EAA) in the alternative ingredients;

$\sum EAA_{fish/soy}$ = sum (%) of EAA in fish meal (average of anchovy, herring, and menhaden) or soybean meal (dehulled, solvent extracted);

CP_{alt} = amount (%) of crude protein (CP) in the alternative ingredients; and

$CP_{fish/soy}$ = amount (%) of CP in the fish meal or soybean meal

Improving Protein quality in Plants

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.

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 etal., 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.

Anti-nutritional Factors (ANFs)

Almost every plant-based alternate protein source has some sort of ANF present (Francis et al., 2001). These compounds are defined as substances that interfere in food utilization and affect the health and production of the animals. There are many different kinds of ANF, such as protease inhibitors, tannins, and lectins which affect protein utilization and digestion; phytates, gossypol, oxalates, and glucosinolates which affect mineral utilization; ant vitamins and miscellaneous factors such as mycotoxins, cyanogens, alkaloids, mimosine, nitrate, saponins, photosensitizing agents and phytoestrogens (Francis et al., 2001). Another negative quality of these plant-based proteins is that they are usually low in palatability, especially when fed to carnivores (Hardy, 1996). Some examples of the plant-based protein sources that contain these ANF are SBM, rapeseed meal, lupin seed meal, pea seed meal, cottonseed meal, sunflower oil cake, leucaena leaf meal, alfalfa leaf meal, mustard oil cake and sesame meal (Francis et al., 2001). Some of these ANF can be eliminated through special treatment such as thermal processing. Heat-labile factors such as phytates, protease inhibitors, lectins, goitrogens and antivitamins can be destroyed by this process (Francis et al., 2001). Other antinutritional factors such as saponins, non-starch polysaccharides, antigenic proteins and estrogens are more heat stable and resistant to thermal processing. A list of plant-based protein sources and their corresponding antinutritional factors can be seen in Table 3.

Effects of Different Processing Methods on Ant nutritional Factors

Seed processing techniques (e.g. soaking, germination, hydrothermal processing, and fermentation) increased cereal and legume enzyme activity. For instance, seed germination resulted in activation or synthesis of phytase and lactic acid fermentation is favourable for cereal phytase activity (Sandberg 2002).

Most of the processing methods employed involve application of heat to eliminate or reduce the level of toxic and inhibitory substances. However, detectable levels of some antinutrients will remain even after thermal treatment (e.g. lectins). Hydrothermal treatments, fermentation and germination have been shown to be most effective in reducing the antinutrients of Mucuna seeds (Wanjekeche et al 2003).

Heat Treatment

In West Africa, seeds require extensive boiling and soaking eliminate some of the toxic constituents before consumption (Carsky et al 1998). Various processing methods have been employed by investigators to reduce the L-DOPA of Mucuna seeds. Egounlety (2003) reported decrease of L-DOPA after pretreatment of Mucuna pruriens var. utilis seeds. Raw Mucuna seeds showed initial L-DOPA up to 6.36%, which was reduced to 4.71% on boiling for 45 min followed by dehulling. Similarly, other treatments also showed significant reduction of L-DOPA (boiling, 45 min + dehulling + soaking, 12 hr reduced L-DOPA to 2.29%; boiling, 45 min + dehulling + soaking, 12 hr + re-soaking, 12 hr reduced to 1.36%; boiling, 45 min + dehulling + soaking, 12 hr + re-soaking, 12 hr + re-boiling, 45 min reduced to 0.64%). Wanjekeche et al (2003) reported that boiling the whole mature seeds of Mucuna pruriens in alkaline solution known as 'Magadi soda' (hydrated sodium carbonate) reduced L-DOPA by 59.3% (5.75% vs. 2.34%), while boiling in cob ash, citric acid and bean stover ash solution reduced it by 58.1, 49.7 and 47.4% respectively (5.75 vs. 2.81, 2.89, 3.02%). Boiling seeds in water or germination up to 5 and 7 days followed boiling, reduced L-DOPA up to 24.9 and 38.5% respectively.

Diallo and Berhe (2003) demonstrated two ways to reduce L-DOPA of Mucuna seeds: (i) Cracking the seeds and soaking them in running water (from a faucet) for 36 hr; (ii) placing whole seeds in a cloth bag and leaving them immersed in a flowing river for three days. The results revealed that cracking Mucuna seeds followed by leaching removed L-DOPA faster than whole seeds. Leaching of cracked and whole seeds in running water via faucet up to 48 hr decreased L-DOPA up to 0.08 and 1.60% respectively (control: whole seeds, 4.93%; cracked seeds, 4.33%). Bressani et al (2003) evaluated the impacts of a variety of processing methods to reduce L-DOPA and trypsin inhibitors of Mucuna seeds. Soaking for 96.5 hr at 22°C resulted in 70% retention of L-DOPA, while the retention decreased to 51% at 45°C and 27% at 66°C after 96.5 hr of soaking, indicates that water temperature plays a significant role in reduction. The L-DOPA was significantly reduced on replacing the soaked water periodically. In white and mottled seeds of Mucuna, soaking and periodically changing water (60°C) resulted in reduction of L-DOPA up to 22-30% of the initial value within 48 hr. Nyirenda et al (2003) studied the effects of different processing methods suitable for household and community level preparations (soaking, boiling, soaking + boiling, with or without sodium bicarbonate) on L-DOPA of Mucuna seeds. Raw seeds possessed initial L-DOPA of 3.75, 3.90 and 4.36% for white, speckled and black

seeds respectively, while pre-soaked speckled beans possessed an initial level of 4.02%. Soaking grits (1.5 l) + boiling (1.5 l) followed by soaking in (1.5 l) for 24 hr in the presence of sodium bicarbonate (0.25%) extracted approximately 90% of L-DOPA (4.02% vs. 0.39%). Similar treatment to whole seeds reduced L-DOPA up to 67%. Soaking grits (24 hr in 3 l water without sodium bicarbonate) reduced L-DOPA up to 54% (4.02 vs. 1.86%). However, in the absence of sodium bicarbonate, boiling the whole seeds and grits without soaking in water reduced L-DOPA only up to 48.5% (4.02 vs. 2.07%) and up to 57% (4.02 to 1.72%) respectively. Bressani et al (2003) have concluded that, even though combinations of boiling, treating with sodium bicarbonate and soaking reduced L-DOPA, boiling alone was the best method for removal in Mucuna seeds. At International Institute of Tropical Agriculture (IITA), Benin, a recipe for the preparation of detoxified Mucuna flour has been developed, wherein L-DOPA was totally absent and the incorporation of these detoxified flours was appreciated by the locals (Versteeg et al 1998).

Various processing methods have been tried by investigators to reduce L-DOPA of Mucuna seeds. Most of the methods employed were based on the use of water, chemicals and thermal treatments (Bressani 2002, Diallo and Berhe 2003, Gilbert 2002). Siddhuraju et al (1996) found dry treatments to be most effective in reducing L-DOPA in Mucuna seeds and attributed the reduction to racemization under roasting. Dossa et al (1998) also showed that grilling was a better technique than cooking in reducing L-DOPA concentration. Garcia Echeverria and Bressani (2006) studied the effects of various cooking treatments (microwave, vapor, in various water solutions at pH 3, 6, 7, 9 and 11 and by cooking in alkaline condition using sodium hydroxide/potassium hydroxide/calcium hydroxide) on the reduction of L-DOPA in Mucuna seeds. Their results indicated that none of the treatments used were effective in eliminating L-DOPA of Mucuna except for calcium hydroxide treatment at pH 9 with washing in hot water (reduction up to 80.4%).

Ukachukwu and Obioha (2000) reported that boiling of Mucuna cochinchinensis up to 90 min (100-105oC) failed to eliminate all of the haemagglutinating activities. Excessive heating of some legumes may ensure the removal of haemagglutinins; unfortunately such practices lower the protein availability as well as protein digestibility (Kakade and Evans 1965). Cooking and autoclaving are known to reduce the hemagglutinating activity up to 89-99% (Vijayakumari et al 1996). Cooking of seeds of Mucuna cochinchinensis up to 3 hr (at 100°C) eliminated the haemagglutinin activity (Onwuka 1997).

Moisture in seeds plays an important role in the destruction of trypsin inhibitors (Liener and Kakade 1980). Udedibie and Carlini (1998) showed that trypsin inhibitors could be completely inactivated in Mucuna seeds on cooking (1 hr, at 96°C). Complete elimination of trypsin inhibitor activity was achieved at 48 hr of soaking in water followed by 30 min cooking. Toasting of the seeds was unsuccessful in complete elimination of trypsin inhibitors, wherein

only 42% eliminated against control (6979 vs. 11865 TIU/g). Antitryptic activity in raw seeds of Mucuna utilis (2170 TIU/g) was totally eliminated on cooking (Ravindran and Ravindran 1988). Bressani et al (2003) showed that germination and malting significantly decrease trypsin inhibitor activity (2 days vs. 6 days; 1.88 vs. 0.82 TUI/mg). They also demonstrated that roasting of Mucuna seeds reduced the trypsin inhibitors significantly (raw vs. 30 min roasting; 18.90 vs. 1.58 TUI/mg). Wanjekeche et al (2003) reported that trypsin inhibitor was reduced up to a greater extent (89.7%) on boiling the seeds in water (27.18 vs. 2.80 TIU/mg). Germination up to 5 and 7 days resulted in reduction of trypsin inhibitors up to 84.5 and 85.4%.

Autoclaving and cooking of pre-soaked Mucuna seeds in different solutions resulted in significant decline in phytate content (27-34% and 38-51%) (Siddhuraju and Becker 2001c). Both dry heat treatment and autoclaving reduced the phytic acid in the seeds of Mucuna pruriens (36% and 47%) (Siddhuraju et al 1996). Soaking in distilled water is also more effective in decreasing phytic acid of Mucuna pruriens seeds than soaking in sodium bicarbonate solution (Vijaykumari et al 1996). Phytic acid was reduced more on soaking seeds in distilled water than sodium bicarbonate solution (27 vs. 17%), following cooking up to 90 min and autoclaving up to 45 min resulted in further decline of phytic acid (18 and 44%).

Cyanide, an antinutritional component of legume seeds can be eliminated on soaking and removal of testa before boiling. The hydrogen cyanide (HCN) is significantly reduced during dry heat treatment (67%) and autoclaving (68%) Mucuna pruriens seeds (Siddhuraju et al 1996). Ravindran and Ravindran (1988) opined that cooking significantly reduce HCN in seeds of Mucuna utilis. Cooking reduces the cyanide content up to 46%, while autoclaving up to 75%. Cooking is a safe method to eliminate toxicity in legume seeds because it destroys the enzyme linamarase at 72°C, but not the glucoside. Most of the liberated HCN was lost through volatilization during cooking and cyanide is rapidly converted to thiocyanides or other compounds (Montgomery 1980).

Siddhuraju et al (2000) reported a significant reduction in total phenolics (up to 80%) in Mucuna seeds by dehulling or by soaking followed by irradiation. Vijayakumari et al (1996) studied the effects of soaking, cooking and autoclaving on some of the antinutritional features of seeds of Mucuna pruriens. Total free phenolics showed significant reduction in soaking sodium carbonate solution (56%) than in distilled water (47%). Autoclaving up to 45 min significantly reduced the tannins (71%). They recorded significant reduction in hemagglutinin activity against human blood groups (A, B and O) through cooking and autoclaving.

Agbede and Aletor (2005) reported the impacts of several methods of processing on the antinutritional features of Mucuna pruriens seed flours. The lectin was completely eliminated by dehulling + cooking and dehulling + roasting than raw seeds (4.0 HU/mg). Autoclaving (raw or dehulling), dehulling + roasting and dehulling + soaking in urea completely removed trypsin inhibition activity of raw seeds (25.3 mg/g). Phytin and phytin phosphorus were highest in raw

Mucuna seeds (15.3 and 4.3 mg/100 g) and lowest in dehulled + roasted seed flours (6.0 mg/100 g). The cyanide content, averaged 18.6 mg/kg in raw seeds was not detected after roasting or dehulling + roasted samples.

Fermentation

El-sayed, (2003) Reports that Utilization of seaweeds and other aquatic plants is also limited due to the presence of high crude fiber and low protein content. Fermentation is a unique process which will improve the nutritional value of feed ingredients. Fermentation reduces the presence of exoskeleton chitin in shrimp head meal, anti-nutritional factors and fibre in plant based feed ingredients thus improves their nutritive value. Further bacterial fermentation hold promise for growth enhancement and immune stimulants in aquaculture. Fermentation also increases the availability of certain vitamins viz., riboflavin, cyanogobalamine, thiamine, niacin, B6, B12 and folic acid levels in some feed ingredients.

Fermentation is an environmentally friendly process consumes less energy and produces less waste. It is a typical example of biodiversity put in to efficient usage that can be applied to a variety of different products. The fermentation process significantly improves nutritive value, acceptability, digestibility and eliminates anti-nutritional factors in plant based ingredients. This provides a promising future for sustainable aquaculture. Fermentation will help feed manufacturers to replace fish meal to certain levels and help in reducing the feed cost and thereby increasing the profitability of aquaculture systems.

Limitations to Plant Proteins

Biological problems associated with the use of alternative plant protein sources must be overcome to effectively increase the use of these products in aqua feeds. They include:

1. Lower CP levels in the replacement ingredient than in the ingredient(s) being replaced.

2. Possible amino acid deficiencies caused by the replacement of high-quality ingredients with substitutes possessing less favorable amino acid profiles.

3. The presence of antinutritional (growth-inhibiting) factors in many of the plant products that has potential as alternative protein supplements.

Conclusion

In conclusion, Problems with fish meal in the world market led many manufacturers of fish feed to look for alternative sources of protein. Feed formulations for farmed fish are expected to change in the future, mainly through a reduction in the percentage of fish meal used to produce grow-out feeds. The extent of these changes will vary depending upon the species of fish, but in general higher percentages of plant proteins will be used in place of fish meal. However, some

suggested ways of overcoming these deficiencies in plant protein is by supplementing the deficient amino acids or by mixing complementary alternatives to obtain the desired essential amino acid profile (Davies et al., 1989; Tacon etal., 1983).

Different processing techniques have to be applied to use underutilized plantsto remove the antinutritional factors.

It is, therefore, necessary to improve nutritional quality and balance essential amino acids and remove toxins and anti-nutritional factors in plants that can be used as source of protein in aquafeeds.

Recommendations

Alternative plant protein sources have to replace the costly fish meal in aqua feeds. Knowledge of the protein content, Amino acid profile and the antinutritional compounds in the intended plant to be used is important.

New and inexpensive means of processing techniques have to be applied to use alternative plants as food, fodder and to exploit the phytochemicals at industrial scale.

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Table 1: Amino Acid Composition of Some Fish Feedstuffs

Ingredients	Ala.	Val.	Gly.	Iso.	Leu.	Pro.	Thr.	Ser.	Met.	Phy.	Tyr.	Lys.	His.	Arg.	Cys.	Asp.	Gla.	Try.	Sources
Fishmeal (Clupeid)	8.26	5.83	8.85	2.62	8.31	4.75	5.50	6.43	2.21	5.50	3.91	11.09	4.28	5.51	-	12.53	18.58	-	Eyo(2001)
Maggot meal	6.1	5.6	5.4	4.13	6.95	3.16	4.53	4.51	2.24	6.95	8.10	7.37	3.5	5.42	0.82	10.8	12.2	1.45	Okoye(2003)
Soybean (Full fatted)	2.72	2.20	3.81	2.13	4.42	3.56	2.59	6.79	1.06	4.35	2.21	3.72	2.55	2.91	-	8.02	14.02	-	Eyo(2001)
Groundnut cake	2..84	2.73	7.49	2.40	6.73	3.47	2.71	5.00	0.61	4.37	3.36	3.23	2.85	11.07	-	9.82	10.74	-	Eyo(2001)
Hydrolysed feather	-	5.96	-	3.76	7.32	-	3.76	-	0.53	3.31	2.33	1.72	0.61	5.90	3.08	-	-	-	Tacon(1987)
Grasshopper	5.29	3.47	4.78	4.21	5.30	5.02	4.08	5.22	1.96	4.50	2.88	5.87	4.24	7.62	1.79	9.32	15.21	-	Okoye(2003)
Dry poultry dung	1.06	0.62	0.82	0.05	0.80	-	0.50	0.52	0.09	0.45	0.26	0.49	0.20	0.47	1.09	1.06	1.54	0.53	El Boushy and Poel (1994)
Blood meal	6.81	4.60	4.09	0.81	10.28	4.61	4.71	9.50	1.57	7.52	2.16	9.19	6.04	2.47	-	8.72	8.40	-	Eyo(2001)

Keys: Ala. (Alamine), Val. (Valine), Gly. (Glycine), Iso. (Isoleucine), Leu. (Leucine), Pro. (Proline), Thr. (Threonine), Ser. (Serine), Met. (Methionine), Phy. (Phenylalanine), Try. (Tyrosine), Lys. (Lysine), His. (Histidine), Arg. (Arginine), Cys. (Cysteine), Asp. (Aspartic Acid), Glu. (Glutamic Acid), Try. (Tryptophan)

Source: Sogbesan (2014)

Table 2. Replacement scores of Selected plant protein products[a].

Ingredient	Fish meal[b]	Soybean meal[b]
Potato protein concentrate	1.58	2.89
Peanut meal	0.50	0.91
Sesame seed meal	0.41	0.74
Safflower seed meal	0.38	0.70
Sunflower seed meal	0.32	0.59
Linseed meal	0.25	0.47
Leucaena leaf meal	0.15	0.28
Fababean seed meal	0.14	0.26
Mustard seed meal	0.14	0.25
Lentil seed meal	0.14	0.25
Pea seed meal	0.11	0.25
Coconut meal	0.10	0.18
Palm kernel meal	0.06	0.11

[a]Replacement Score = ($\sum$EAAalt /$\sum$EAAfish/soy) X (CPalt /CPfish/soy), Where $\sum$EAAalt = sum (%) of essential amino acids (EAA) in the alternative ingredients; $\sum$EAAfish/soy = sum (%) of EAA in fish meal (average of anchovy, herring, and menhaden) or soybean meal (dehulled, solvent extracted); CPalt = amount (%) of crude protein (CP) in the alternative ingredients; andCPfish/soy = amount (%) of CP in the fish meal or soybean meal.

[b]Scoresin each column represent the relative value of an ingredient as a 1:1 replacement for fish meal or soybean meal, based on the ingredients amino acid composition and protein content. A score of 1.0 or higher suggests that the ingredient could a suitable replacement for fish meal or soybean meal with little or no modification. Scores below 1.0 indicate the presence of amino acid deficiencies that should be corrected (e.g., amino acid supplementation) before the ingredient is used as a primary protein source.

Source: (Reigh, 2008)

Table 3: Nutritive Values of some Animal and Plant Protein Feedstuffs (% dry matter)

Feedstuffs	CP%	CL%	CF%	Ash%	NFE%	DM%	P%	Ca%	G.E Kcal/100g	Sources
Fishmeal(Clupeids)	71.3	7.97	1.08	20.22	-	90.2	-	-	-	Eyo (2001)
Blood meal	86.0	0.67	2.14	6.43	6.51	88.2	0.39	0.55	-	Eyo (2001)
Soybean meal	46.2	24.8	4.7	7.9	20.7	91.0	0.76	0.22	-	Eyo (2001)
Groundnut cake	43.5	23.4	6.0	6.2	19.1	91.0	0.37	0.22	-	Eyo (2001)
Meat and bone meal	46.0	10.0	2.0	-	-	-	5.5	10.7	-	Faturoti (2003)
Hydrolized feather meal	84.0	2.5	1.5	-	-	-	0.8	0.2	328.6	Faturoti (2003)
Poultry feather meal	39.7	84.9	0.9	26.9	1.4	3.5	-	-	-	Tacon (1993)
Dry poultry dung	24.5	8.42	13.2	20.1	21.9	12.3	-	-	305.6	Fasakin et al (2000)
Maggot live	52.0	11.2	2.1	16.4	6.1	87.8	-	-	-	Madu and ufodike (2003)
Earthworm	56.7	7.8	1.6	8.8	25.4	11.7	0.5	0.9	-	Tacon (1994)
Tadpole	43.5	11.3	-	34.75	10.45	-	-	-	-	Ayinla et al. (1994)
Alate (edible)	32.0	42.6	5.2	-	-	-	-	-	538.9	Adiku (1993)
	44.2	22.2	7.31	7.34	19.0	87.41	-	-		Fadiyimu et al (2003)
Snail meal	66.5	8.0	-	8.3	94.3	-	-	-	-	Seira (1998)
Whole grasshopper meal	57.6	7.24	10.56	13.6	-	-	-	-	-	Okoye (2003)
Yellow maize	10.1	3.56	3.47	1.94	71.2	90.4	0.28	0.15	-	Eyo (2001)
Freshwater mussel	24.9	2.00	9.00	32.74	21.61	90.4	0.28	0.15	-	Ojo (2003)
Azolla Africana (aquatic fern)	28.9	4.6	12.2	15.3	47.6	89.6	0.7	2.78	-	Fasaki et al (2001)
Spirodela polyorhiza (Duckweed)	25.6	4.2	8.7	15.2	46.6	91.7	0.5	9.63	-	Fasaki et al (2001)

Keys: CP-Crude Protein, CL- Crude Lipids, CF- Crude Fibre, NFE- Nitrogen free- extract, DM- Dry Matter, P- Phosphorus, Ca- Calcium, GE- Gross Energy.

Source: Sogbesan (2014)

Table 4. Alternative protein ingredients and their corresponding antinutritional factors

Alternative Protein Ingredient	Antinutritional Factor
Soybean meal	Low palatability, protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens, low in methionine
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins, high fiber
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamins, cyclopropenoic acid
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Corn Gluten meal	High fiber, presence of cartenoids can turn the flesh of fish to a yellowish color
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins
Wheat Gluten	Expensive
Poultry by-product meal	Variable quality, high ash content

Source: (Lungeret al. 2006)

Table 5—. Effects of various processing methods on antinutrients of some underutilized legumes.

Legume	Processing	Observation	Reference
A. precatorius	Soaking, cooking, autoclaving, and roasting	Autoclaving was found to significantly reduce various antinutrients	Pugalenthi and others (2007)
African yam bean seeds	Boiling and solid substrate fermentation	Boiling of seeds resulted in 8% to 30% reduction of total α-galactosides, while in the modified tempeh, α-galactosides reduction was by 22% to 39%	Azeke and others (2007)
B. purpurea	Soaking, cooking, and autoclaving	Soaking in distilled water caused maximum reduction in the phytic acid (37%), whereas soaking in $NaHCO_3$ solution reduced phenolics and tannins (by 72% and 78%, respectively)	Vijayakumari and others (2007)
		Cooking resulted in the reduction of oligosaccharides (raffinose by 63%, stachyose by 42%, and verbascose by 79%)	
B. Eurycoma, *D. microcarpum*, and *Mucuna sloanei*	Roasting, boiling, dehulling, shelling followed by soaking	Reduction in phytic acid and polyphenol contents	Giami and Wachuku (1997)
C. ensiformis	Pre-soaking seeds of *C. ensiformis* in kitchen soda (10 g/l; 1 : 3 w/v)	Reduction in the canavanine (75% to 82%)	Gupta and others (2001)

Table 5—. Effects of various processing methods on antinutrients of some underutilized legumes.

Legume	Processing	Observation	Reference
Canavalia cathartica (dried seeds)	Thermal processing (pressure cooking and roasting)	Decrease in total phenolics and hemagglutinins	Seena and others (2006)
Canavalia cathartica (ripened beans)	Pressure-cooking	Phytohemagglutinin activity of raw beans decreased to about 50% on cooking	Bhagya and others (2007)
Phaseolus lunatus, *Sphenostylis sternocarpa*, and *C. ensiformis*	Soaking, cooking, and germination	Raffinose oligosaccharides were reduced after all the processing, while germination and cooking in alkaline medium caused the greatest losses of total α-galactosides	Oboh and others (2000)
P. angularis and *P. calcaratus*	Soaking for 12 and 18 h; cooking, autoclaving, and germination for 24 and 48 h	Reduced levels of antinutrients including phytates, tannins, trypsin inhibitors, and amylase inhibitors	Chau and Cheung (1997)
Prosopis chilensis (Molina) Stunz.	Soaking, cooking, and autoclaving	Distilled water and sodium bicarbonate solution (salt water) soaking significantly reduced total free phenolics.	Vijayakumari and others (1997b)
		Cooking and autoclaving significantly reduced the phytic acid content (32% to 35%).	
		Autoclaving (45 min) reduced the raffinose and stachyose (63% to 71%).	
Sesbania (*S. aculeata*, *S. rostrata* and *S. cannabina*)	Aqueous soaking followed by gamma irradiation (2, 4, and 6 kGy)	Irradiation significantly increased the total phenolic contents, while no significant effects were recorded in the phytic acid and canavanine contents.	Siddhuraju and others (2002)
		Nearly 50% reduction of lectin activity was observed (at 6 kGy). Irradiation of *S. aculeata* significantly reduced the trypsin inhibitor activity	
Mucuna pruriens L.DC.	Gamma irradiation	Increase in phenolics, with complete degradation of phytic acid (at 15 and 30 kGy), L-dopa concentration showed a dose-dependent decline. Trace amount of hemagglutination activity seen on human erythrocytes was completely absent on irradiation (>5 kGy)	Bhat and others (2007b)
Mucuna pruriens (L.) DC. var utilis (Wall ex Wight) (velvet bean)	Soaking in tap water, soaking in alkali/acid solutions, soaking followed by cooking, soaking, autoclaving, microwave cooking, germination, dry heating, and gamma irradiation	Germination (120 h) and soaking in Ca (OH)$_2$ solution resulted in significant reduction of L-dopa and gamma-irradiation at 10 kGy was more effective in reducing the L-dopa	Gurumoorthi and others (2008)

Source: (Rajeev et al., 2009)