POTENTIALS OF LEAF MEAL AND THEIR PROTEIN CONCENTRATE IN AQUAFEED

Olude O. O.[1] and N. P. Sahu[2*]

[1]*University of Lagos, Lagos, Nigeria*

[2]*ICAR-Central Institute of Fisheries Education, Mumbai- 400 061 India*

**Corresponding author: npsahu@cife.edu.in*

The quest to sustain current aquaculture growth trend has necessitated the search for alternative feed ingredients in the light of conventional ones whose supply have dwindled and have become prohibitive. A major group of under-utilized, renewable, abundant and cheap unconventional source is leaf meal. They can be derived from the vast array of terrestrial and aquatic plants; most of which are nutrient-rich with little or no direct relevance in human nutrition. The nutritional composition of leaf meals usually varies widely; depending on plant cultivars from which they are derived, soil and climatic conditions, richness of medium in which they grow in case of aquatic plants, age and maturity of plants among other factors. Their limitation in fish nutrition is usually improperly balanced amino acid profile with methionine and lysine often limiting; nutritional-stress factors such as tannins, phytic acid, protease inhibitors among others; high fibre and palatability. The significance of these limitations is evident in impaired growth and reduced digestibility. Several efforts such as soaking, sundrying, fermentation, enzyme or amino acid supplementation have proven to be useful in improving the nutritional composition of leaf meals such that they are incorporated in aquafeed at higher inclusion levels relative to their raw state. Production of proteins from green leaves provide means of reducing antinutrients and high fibre contents associated with leaf meals while also providing a rational and sustainable strategy of sharing leaf resources among ruminants and non-ruminants. The technology of leaf protein extraction from green leaves may be increasingly relevant in future aquafeed if the drawbacks associated with its production are addressed. Further empirical information through intensified and co-ordinated research on the utilization of available leaf meals, either as protein or energy source, is needed to ensure feed security in aquaculture production.

INTRODUCTION

Fish has become the major source of animal protein in human diet as evident in the progressive increase in its per capita consumption which rose from 9.9 kg in 1960s to its current level of above 20 kg per annum (FAO, 2016). In this regard, the importance of aquaculture in total fish supply to compensate for the dwindling wild fish stock that has been fully or over-exploited has been recognized (FAO, 2016). Aquaculture is growing more than every other animal food producing sector and its fishcomponent reached 76.6 million tonnes in 2015 (FAO, 2017). This growth trend is expected to continue as the World Bank (2013) estimated that aquaculture will supply 93.2 million tonnes of fish by 2030. The growth in aquaculture production has been attributed to increased and improved feed inputs (Naylor *et*

al., 2009) especially among farmers in China and other Asian countries (particularly Indonesia and India) who are the leading global producers (FAO, 2016). Projected increase in fish production will therefore mean production of more quality feed; more so since feed represents about 60% of the inputs in the farming of most fish species (Gabriel *et al*., 2007; FAO, 2017). In other words, if suitable feed sources are not available in the right quantity, the projected growth of aquaculture will not be realized; a situation which will have dire consequences on the global supply of animal protein especially to the teeming poor. In 2015, global feed production for farmed fish and crustaceans was approximately 47.7 million tons, excluding commercial feed consumed by Indian major carp (FAO, 2017) and based on predicted aquaculture growth it is expected to reach 77.93 million tons by 2020 (Grand View Research, 2014 (http://www.grandviewresearch.com/press-release/global-aquafeed-market).

The conventional protein ingredients such as fish meal and soybean on one hand; and energy sources such as maize and rice bran used in aquafeed industry are becoming prohibitive as a result of competition with other uses. Substantial research efforts expended on the reduction of fishmeal component of fish feed over the years have yielded great results both in carnivorous and herbivorous species (Hardy, 2010). Nonetheless, huge quantity of fishmeal is still being used in aquaculture sector as a result of its expansion; instigating the need for more research to replace fishmeal especially in the diets of fish fry and fingerlings (Tacon and Metian, 2008; Hardy, 2010). Similar context is also applicable in the use of soybean, maize and rice bran which are directly being used in human nutrition, livestock production and various industrial applications (Alegbeleye *et al*., 2012; Al-Shorgani *et al*., 2012) making demand for these commodities to outweigh supply for which reason they have become prohibitive.This scenario, necessitating the search for alternative feed ingredients, has been exacerbated by the factor of climate change. Hall (2015) reviewed the impacts of climate change on aquaculture and emphasized the need for alternative feed ingredients in the aquafeed industry in view of the consequences of climate change evident in increased temperature, altered rainfall patterns and drought among others; making irrigation problematic and impacting negatively on production of major crops like rice, maize, soybean, wheat etc which apart from being directly consumed by humans are also used (directly or indirectly) to a large extent in animal feed industry. Similarly, climate change has been identified as factor impacting on the abundance and distribution of fisheries from which fishmeal is derived leading to its uncertain supply. Accordingly, for aquaculture to sustain its current growth trend and attain its full potential in contributing to the supply of animal protein for the increasing global population there is an urgentneed to develop less competitive but qualitative feed and feed ingredients, that will not compromise product quality, for the aquafeed industry.

MERITS OF LEAF MEALS AND THEIR SOURCES

One possible source of alternative to conventional aquafeed ingredients is leaf meal because they are abundant, renewable and can be sustainably produced to keep pace with the

growth of aquaculture industry. Plants occupy the base of the food chain both in the terrestrial and aquatic ecosystem and can synthesize amino acids from virtually unlimited and readily available primary materials; for which reason they are cheap and abundant (Stahmann, 1968; Fasuyi and Aletor, 2005). Leaf meals are nutrient-rich albeit with highly varied crude protein content depending on plant species and cultivars from which they are derived, soil and climatic conditions, age and maturity of plants among other factors (Table 1) (Akeson and Stahmann, 1966; Bairagi *et al.*, 2002; Oresegun *et al.*, 2016). They have a good balance of minerals and are rich in vitamins (Ayssiwede *et al.*, 2011). Several studies have demonstrated their potentials as alternative feedstuff in fish feed (Osman *et al.*, 1996; Bairagi *et al.*, 2002; Adewolu and Adamson, 2011). Many leaf meals also have medicinal properties which have been shown to influence immune system positively (Priyadharshini *et al.*, 2011; Talpur and Ikhwanuddin, 2013; Fawole *et al.*, 2013). They can be sourced from vast varieties of terrestrial and aquatic plants (Table 1) which are sub-classified as tree leaves, crop by-products, leafy vegetables and aquatic weeds for the purpose of this review.

Tree leaves

Ligneous plants, which may be trees, small trees, shrubs and under shrubs have been traditionally used as important component of fodder resources for livestock and wildlife; especially during the dry season when the available grazing is not generally sufficient to meet the maintenance requirements of animals (FAO, 1992; Habib *et al.*, 2016). A large percentage of tree leaves are legumes which have high forage yields with high crude protein content (12-30%, FAO, 1992; Habib *et al.*, 2016); they grow in poor soils and are drought-tolerant. In view of their nutritional composition and abundance at relatively reduced cost, their role in monogastric nutrition has been recognized. Information on the nutritional composition and feed utilization of *Moringa spp, Gliricidia spp, Leucaena leucocephala* among other tree leaves in monagastric nutrition are well documented. The results of these studies vary but indicated that reasonable levels of tree leaves can be utilized in fish, poultry and pig diets especially when factors limiting their utilization are addressed (Osman *et al.*, 1996; Richter *et al.*, 2003; Kambashi *et al.*, 2014). Perhaps the most researched tree leaves in fish feed are those of ipil-ipil leaf (*Leucaena leucocephala*) and *Moringa spp*. In a trial assessing growth performance and nutrient utilization, Richter *et al.* (2003) incorporated graded levels (10, 20 and 30%) of freeze-dried moringa (*Moringa oleifera*) leaf meal in Nile tilapia, *Oreochromis niloticus*, diet and observed a significantly reduced growth performance and nutrient utilization when the leaf meal was added beyond 10%. They attributed the inferior performance in the group fed higher percentages (20 and 30%) of moringa to poor feed intake at the initial stage of the experiment, high levels of saponins, total phenolics, phytic acid, neutral and acid detergent fibre. Similarly, a 10% inclusion level of moringa (*Moringa oleifera*) leaf meal was recommended in the diets for *Clarias gariepinus* when replacing fish meal (Ozovehe, 2013) and 20% when replacing soybean (Ncha *et al.*, 2015) without compromising growth. Olude and Badmus (2015) evaluated mixture of moringa leaf and kernel meals as partial replacement for fishmeal in the diet of *Clarias gariepinus* juveniles and concluded that up to 20% of the mixture could be used

Table 1: Crude protein (%), Crude fibre (%) and Antinutrients of some selected leaf meals

Leaf meal	Crude protein	Crude Fibre	Antinutrients	Source
Leucaena leucocephala	20.4-27.3	6.2-12.1	Mimosine, Tannin, Phytic acid	Bairagi *et al.* (2004); Reyes and Fermin (2003); Aye and Adegun (2013)
Moringa spp	20.0 -26.6	6.8-8.8	Phenolics, Tannins, Phytic acid, Saponins; Trypsin inhibitor	Richter *et al.* (2003); Reyes and Fermin (2003); Aye and Adegun (2013)
Gliricidia spp	15.6-25.1	8.6-13.8	Tannin, Phytic acid, Oxalate, Saponin	Amata and Bratte (2008); Aye and Adegun (2013)
Albizia spp	18.1-22.2	19.8-26.8	Phytate, cyanide, Oxalate, Saponin, Tannin	Habib *et al.* (2016); Chitra and Balasubramanian (2016)
Trichanthera gigantea	11.5-23.5	30.0-56.7		Leterme *et al.* (2005)
Centrosema	11.5-23.2	8.8-23.0	Phytic acid, Tannin	Nworgu and Egbunike (2013); Agbede (2006)
Mucuna pruriens	21.4-27.0	10.0-12.0	Phytic acid, Tannin, Oxalate, Saponin	Agbede (2006); Sese *et al.* (2013)
Delonix regia	10.9	13	Phytic acid, Tannin	Agbede (2006)
Acacia spp	13.6-14.2	21.8	Phytic acid, Tannin	Agbede (2006); Akinyemi and Kayode (2012)
Carica papaya	23.0-30.1	11.4-14.1	Saponin, Phytic acid, Tannin, Cyanide	Reyes and Fermin (2003); Paranamana *et al.* (2015)
Morus alba	11.3-23.5	8.7-38.4	Phytic acid, Cyanide, Tannin	Leterme *et al.* (2005); Adeduntan and Oyerinde (2010)
Glycine max	16.9-27.50	9.56	Saponin, Phytic acid, Tannin, Cyanide	Paranamana *et al.* (2015); Nkosi *et al.* 2016
Ipomoea batata	20.5-23.6	8.3-8.4	Saponin, Phytic acid, Tannin, Cyanide	Adewolu (2008); Paranamana *et al.* (2015)
Lemna spp	7.0-40.0	5.0-30.0	Trypsin inhibitor, Cyanogens, Tannins, Gossypol	Leng *et al.* (1992); Islam (2002)
Azolla pinnata	21.4-27.5	11.6-12.7	Trypsin inhibitor, Phytic acid, Saponins, Tannins	Reyes and Fermin (2003); Gangadhar *et al.* (2015)
Pistia stratiotes	15.96	11.08	Trypsin inhibitor, Saponins, Tannins	Nisha and Geetha (2017)
Eichhornia crassipes	10.4-31.3	16.5-20.4	Trypsin inhibitor, Saponins, Tannins, cyanogens, Phytic acid	Igbinosun and Talabi (1982); Mako *et al.* (2011); Mohapatra (2015); Sotolu (2013)
Manihot esculenta	14.0-40.0		Cyanide, Tannins, Phytic acid	Morgan and Choct (2016)
Vigna unguiculata	22.0-30.0	14.3-25.1	Tannin, Saponin, Phenol	Chikwendu *et al.* (2014); Gonçalves *et al.* (2016)
Arachis hypogea	22.3-29.1	18.6-41.7	Oxalate, Tannins	Almazan and Begum (1996); Garduno-Lugo and Olvera-Novoa (2008)
Vernonia amygdalina	31.7-34.5	8.8-9.1	Oxalate, Phytate, Tannin, Cyanide, Saponin	Aletor *et al* (2002); Shokunbi *et al.* (2011)
Solanum africana	34.5	7.4	Oxalate, Phytate, Tannin, Cyanide, Saponin	Aletor *et al* (2002)
Amaranthus spp	7.6-32.3	7.4-8.8	Oxalate, Phytate, Saponin, Tannin	Fasuyi *et al.* (2008); Adewolu and Adamson (2011); Andini *et al.* (2013); Ngugi *et al.* (2017)
Telfairia occidentalis	22.4-39.4	9.8-14.7	Oxalate, Cyanide, Tannin, Phytate	Akwaowo *et al.* (2000); Aletor *et al* (2002); Fasuyi (2006)

without growth and nutrient utilization compromise. Furthermore, Tekle *et al.* (2015) evaluated the antioxidative and antimicrobial activities of solvent extracts of different parts (leaf, flower, bark, stem and pod) of *Moringa oleifera* in an *in-vitro* study and observed considerable activities in all the extracts which were highest in the leaf and flower. They recommended extracts from leaf and flower as prophylactic agents against oxidative stress and infectious disease.

In his review, El-Sayed (1999) observed a conflict in the results of investigations incorporating leucaena leaf meal in tilapia diet. For instance, Pantastico and Baldia (1979, 1980) stated that growth of *Oreochromis niloticus* and *O. mossambicus* were not compromised when fed diets containing 100% leucaena leaf meal while Jackson *et al.* (1982) and Wee and Wang (1987) reported a significant lower growth and feed efficiency when 25% level of leucaena leaf meal were fed to *Oreochromis niloticus.* It is well known that leucaena leaf meal is deficient in some key essential amino acids and also contain mimosine, a non-protein amino acid that could depress growth (Osman, *et al.* 1996). *Gliricidia spp* is an all-year-round, fast-growing tree legume that offers the potential for utilization in aquafeed because of its large quantity of high quality forage. Vhanalakar and Muley (2014) had reported that *Cirrhinus mrigala* fingerlings utilized *Gliricidia maculata* meal up to 40% in the diet without any adverse effects on growth and feed utilization. Other tree leaves such as *Sesbania spp* (Firmani *et al.*, 2015), *Carica papaya* (Paranamana *et al.* 2015; Raja *et al.*, 2017), *Acacia spp* (Mondal and Ray, 1999) among others have also been characterized and incorporated in fish diets with varying level of success.

Crop residue/by-products and leafy vegetables

Another possibility in the quest of searching for alternative ingredients in aquafeed is the use of leaf residues or by-products of seed and root crops, leafy vegetables and grasses. Substantial quantity of leaves generated as by-products of crops such as cassava, cowpea, groundnut among many others could be incorporated into fish feed at minimal cost. For instance, cowpea forage yield can reach as high as 8 t DM/ha in irrigated areas while periodic harvest of up to 0.4 t/ha of cowpea leaves with no noticeable reduction in seed yield have been reported (Heuze *et al.*, 2015). Imungi and Potter (1983) had reported an average crude protein content of 33.6% for cowpea leaves which were also noted to be rich in minerals. Cassava leaf can yield approximately two tons of dry matter per hectare (Coldebella *et al.*, 2013; Oresegun *et al.*, 2016) with crude protein varying between 14-40% depending on the cultivars, harvesting time, climatic conditions among the other factors. Cassava leaf can be harvested starting from 4 to 5 months of planting and a frequency of 2 to 3 months in 12-month cultivars without having any adverse effects on the roots (Morgan and Choct, 2016) and the crude protein, amino acid and cyanide contents of cassava leaf meal have been reported to decrease as the leaf matures while crude fibre and hemicellulose contents increase (Ravindran, 1991).

Iheukwumere *et al.* (2007) evaluated cassava leaf meal in broilers diet and recommended a dietary level of 5%, beyond which there was deleterious effect on growth,

blood chemistry and carcass yield. They suggested high fire content (11.4%) of the leaf meal resulting in insufficient consumption (low feed intake) of digestible nutrients (particularly protein and energy) could have been responsible for depressed growth at higher levels (10 and 15%) of inclusion. They also opined that cassava leaf meal could have imparted unpalatable taste to the feed and consequently preventing the birds from consuming adequate quantities at higher inclusion levels. It however seems that fish could utilize cassava leaf meal better than poultry as Nieves and Barro (1996) reported a good performance in Nile tilapia when the inclusion level of cassava leaf in their diet did not exceed 15%. Sutriana (2007) observed that poor methionine availability and the presence of cyanide content limited the use of cassava leaf meal in African catfish (*Clarias gariepinus*) fry diet. The possibility of partially replacing fish meal protein with peanut (*Arachis hypogaea*) leaf meal, another abundant crop by-product, in tilapia diet was demonstrated by Garduno-Lugo and Olvera-Novoa (2008).

Green leafy vegetables are also receiving considerable attention because of their economic and nutritional advantage. Many leafy vegetables can tolerate biotic and abiotic stress and have a short growing season, between 20-30 days after transplant, and then every 2-3 weeks for a period of 1-2 months (Maundu, 1997; Fasuyi *et al*., 2008). Aletor *et al.* (2002) researched on the chemical composition of four leafy vegetables (*Vernonia amygdalina, Solanum africana, Amaranthus hybridus, Telfaria occidentalis*) and reported average crude protein, crude fibre and gross energy of 33.3 g/ 100g dry matter, 8.4 g/ 100g dry matter and 378 Kcal/ 100g, respectively. Although, leafy vegetables are mostly collected from the wild where they grow as weed; they are fast-growing when cultivated and can grow on wide range of soil and agro-climatic conditions (Jaarsveld *et al*., 2014). Fasuyi *et al.* (2008) evaluated *Amaranthus cruentus* leaf meal as an alternative protein supplement in broiler starter chicks and concluded that the vegetable could be a useful dietary protein source at 5% inclusion level. Similar result was also observed by Adewolu and Adamson (2011), who tested *Amaranthus spinosus* leaf meal as a potential dietary protein source in practical diets for *Clarias gariepinus*. They attributed low utilization of the leaf meal to nutritional stress factors such as saponins, alkaloids, phenols and oxalates.

Aquatic weeds

Aquatic weeds are widely distributed in freshwater, brackish water and marine environment. They are major distinct group having a high potential as alternative to conventional protein and energy sources in animal feed industry as a result of their profuse growth in natural and impounded water bodies. Often times, they are seen as menace in aquatic environment because they interfere with beneficial usage of water such as transportation, fishing and fish culture; necessitating control measures at some cost (Boyd, 1968). Their nutritional composition depends on the richness of medium in which they grow. For example, Tan (1970) reported that aquatic weeds harvested from natural waters have lower crude protein content relative to those raised in enriched waters containing mineral media, agricultural or domestic effluents. Aquatic weeds have a good balance of amino acid

profile (Dewanji *et al*., 1997; Islam, 2002) high concentrations of trace minerals and pigments such as xanthophylls and carotene (Yan *et al*., 1999) and most species contain low levels of fibre when compared to terrestrial plants (Little, 1979); making them desirable alternatives in monogastric nutrition. Attempts have been made to use several aquatic plants such as duckweed (mostly *Lemna* spp),water fern *(Azolla* sp.*),* water lettuce (*Pistia stratiotes)*, water hyacinth (*Eichhornia crassipes)* among others, as protein source in fish feed. The results of these studies generally show that they could be incorporated in fish feed at levels between 20-40% depending on the level of fish meal in the diets without eliciting a negative growth consequence. For instance, Nisha and Geetha (2017) recently observed that water lettuce (*Pistia stratiotes)* can optimally replace 30% of fish meal in diet of *Labeo rohita,* 40% inclusion of *Nymphaea* leaf meal was recommended by Sivani *et al* (2013) for *Cyprinus carpio* while Naegael (1997) found that up to 30% of fish meal based diet fed to Nile tilapia could be successfully replaced with dried Azolla meal.

Duckweed is another well known, nutrient-rich aquatic plant that has immense potential as alternative feed ingredient in fish feed. Its relevance in animal nutrition has been well detailed in the review of Leng *et al*. (1992) and Islam (2002). Duckweed has four genera namely: *Spirodela, Lemna, Wolffiela* and *Wolffia*; and about 40 species with global distribution (Culley *et al.,* 1981). A major consideration in choosing an alternative feedstuff is easy availability and fast growth. Duckweed reproduces by vegetative propagation and form large masses or colonies which are distributed as a sheet or film on water surface (Leng *et al*. 1992; Islam, 2002). They can double their biomass in a period of two or three days under favourable environmental conditions gaining 10-13tons dry matter/hectare/year in small lakes systems while yielding up to 20 tons dry matter/hectare/year in outdoor tanks (Islam, 2002). The crude protein content of duckweed can range from 7-40%, depending on the media of culture. Leng *et al*. (1995) noted that duckweeds grown in water with 10-30 mg NH_3-N/litre have high protein content (around 40%) of high biological value.The essential amino acid profile of duckweed is similar to other animal proteins with the exception of methionine which is limiting; their crude fibre and mineral contents increase with age (Islam, 2002). Yilmaz *et al*. (2004) studied duckweed (*Lemna minor*) as a protein feedstuff in practical diets for common carp fry and observed that a diet consisting of up to 20% duckweed (substituting commercial feed which has 32% crude protein) could be used as a complete replacement for commercial feed in diet formulation for common carp. Arrivillaga (1994) and Essa (1997) had earlier reported that *Wolffia* and *Lemna*, respectively, replaced up to 50% of commercial feeds (35% crude protein) for Nile tilapia without adverse effects on fish growth and body composition.

Water hyacinth (*Eichhornia crassipes*) is a significant aquatic weed because of the way it proliferates; spreading rapidly and constituting threat to man and the aquatic ecosystem. Huge efforts have been expended over the years to control its menace in the aquatic environment. Although, documented research findings have indicated that water hyacinth has great potential as animal feed source because of its reasonable amount of crude protein (11.3-16.5% dry matter) but its high level of crude fibre (11.7-20.4%) is its major

limitation (Igbinosun and Talabi, 1982). Mohapatra (2015) achieved 40% replacement of fish meal corresponding to 204.8 g/ kg dietary water hyacinth without significant reduction in growth performance of common carp, *Cyprinus carpio.*

LIMITATIONS OF LEAF MEAL

From the foregoing, it is evident that efforts to incorporate the available, renewable and more sustainable green plants at very high level in aquafeed have been constrained by a number of factors. These constraints include deficiency of key amino acids such as lysine, methionine and tryptophan (Table 2) (Wee, 1991), low digestibility (Garduna-Lugo and Olvera-Novoa, 2008), the presence of high amounts of crude fibre and other antinutritional factors such as protease inhibitors, alkaloids, tannins, lectins and adverse oligossacharides (Oresegun *et al*., 2016), decreased palatability as a result of presence of compounds that are offensive to olfactory receptors of fish (Amisah *et al*. 2009). The significance of antinutrients in fish feed has been extensively reviewed by Francis *et al.* (2001). Antinutrients interfere with food utilization and affect the health and production of animals. Tannins are known to interfere with the digestive processes either by binding the enzymes or by binding to feed components like proteins or minerals (Francis *et al.,* 2001). They also impact a bitter, astringent taste which makes leaf meals unpalatable (Makkar, 1993). Phytic acid chelates di and trivalent mineral ions such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{3+} and Fe^{3+}, thereby reducing their digestibility in animals (Riche and Brown, 1996). They also reducedigestibility of protein through formation of phytate-protein complexes, decreased growth performance and inhibit activities of some digestive enzymes suchas pepsin, trypsin and alpha-amylase (Robaina *et al.*, 1995)thereby modifying digestion processes which could impair intestinal absorption. Oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce an acute metabolic calcium deficiency syndrome (hypocalcemia) when fed as the main feed to animals (Checke and Shull, 1985). Saponins are known for their haemolytic activity and ability to depress growth in herbivore (Francis *et al*., 2002). Trypsin inhibitors lead to reduced protein digestibility as a result of irreversible trypsin enzyme-trypsin inhibitor complexes (Vadivel and Pugalenthi, 2009). Cyanide inhibits several enzyme systems through cytochrome oxidase, depresses growth through interference with certain essential amino acids and impairs utilization of associated nutrients (Tewe and Egbunike, 1992; Okafor, 2004 in Lukuyu *et al*., 2014). Mimosine, a non-protein amino acid, acts by interfering in cellular mitosis; causing reduced appetite, reduced weight gain and death. (Osman *et al*.,1996)

The fibre content in leaf meals is also a major concern especially in most monogastrics that do not have cellulase activities in their gut and thus do not have capacity to hydrolyze cellulose which is the major plant constituent (Saha and Ray, 1998). Dietary fibre consists of non-starch polysaccharides (NSP) together with lignin, protein, fatty acids, waxes etc (Bach-Knudsen, 2001). Sinha *et al.* (2011) detailed the factors responsible for anti-nutritive effects of non-starch polysaccharides as modulation in digesta viscousity, alteration in gastric emptying and rate of passage, alteration of gut physiology and morphology, native

gut microflora and mucus layer of gut. These factors are known to delay intestinal absorption of glucose, reduce amino acid digestibility, decrease lipid utilisation, induce hypocholesterolaemia and decrease mineral absorption.

DE-LIMITING LEAF MEALS FOR BETTER UTILIZATION

Several research efforts have been expended in assessing the utilization of many leaf meals in their raw and processed form. The results have always suggested that when leaf resources are processed, their composition in terms of nutritive value is enhanced and can be incorporated at higher percentage than in their raw forms. Accordingly, if there is going to be possibility of extensively increasing the utilization of leaf meals from the numerous available sources in aquafeed, current research efforts must focus more on the development of simple, low-cost, effective and efficient techniques aimed at improving their nutritional composition, reducing their anti-nutrients and increasing their utilization by fish.

Heat treatments

Heat treatments such as cooking, sun-drying or oven-drying (Osman *et al.* 1996; Bradbury and Denton, 2011) have been used by many investigators to reduce the level of plant antinutrients. Heat treatments destroy naturally occuring heat labile factors like protease inhibitors, phytates and lectins (Francis *et al.,* 2001). Osman *et al.* (1996) had observed that drying or cooking of leucaena leaf meal incorporated in Nile tilapia diet significantly improved growth performance and nutrient utilization. Adewolu (2008) had also reported on the potentials of sundried sweet potato (*Ipomoea batatas*) leaf meal as dietary ingredient for *Tilapia zillii* fingerlings and attributed palatability as observed in diet acceptability to the processing used.

Soaking

Soaking has been known to reduce antinutrient from plant proteins either singly or in combination with other processing methods. The review of Kumar *et al.* (2011) pointed out that soaking could hydrolyse phytate at high temperature (45-65°C) and slightly acidic pH (5-6). Hasan *et al.* (1990) studied the suitability of soaked and unsoaked leucaena and water hyacinth leaf meals, as partial substitutes for dietary fish meal protein for fry of *Labeo rohita* and stated that diets containing higher inclusion (40%) of soaked leucaena and water hyacinth leaf meal proved to be better than control and other diets in terms of cost of feed and economic return. Soaking and sun-drying increased palatability of leucaena leaf meal such that it replaced 30% fish meal in *Clarias gariepinus* diet without affecting growth (Amisah *et al.*, 2009). It should, however, be noted that soaking could also results in leaching of minerals, water-extractableproteins and vitamins (Vadivel and Pugalenthi, 2009).

Fermentation

Fermentation technology is an affordable low-energyprocessing technique that could be used to enhance the quality of plant feed ingredients.This technology involves invading food substrates with edible microorganisms whose enzymes, particularly

amylases, proteases, and lipases, hydrolyze the polysaccharides, proteins, and lipids to non-toxic products with flavors, aromas, and textures that are pleasant (Steinkraus, 2009). Mondal *et al*. (2012) replaced fish meal with fermented mulberry leaf meal in the diet for Indian minor carp, *Labeo bata*, and observed that fermentation improved the nutritional quality of the leaf resource to the extent that 50% replacement of fish meal was possible. Saha and Ray (2011) observed reduction in crude fibre, cellulose and hemicelluloses contents and anti-nutritional factors (tannins and phytic acid) of water hyacinth consequent of fermentation using two strains of fish intestinal bacteria (*Bacillus subtilis* and *B. megaterium*). They further reported that the fermented meal elicited good growth when incorporated up to 40% level in the diet for *Labeo rohita.* Wee (1991) noted that loss of nutrient as a result of leaching, destruction by light, heat or oxygen, or microbial utilization during fermentation is commonly small and that there may be an increase in the nutrient level through microbial synthesis; making it a particularly desirable technique in improving plant-derived meals.

Enzyme Supplementation

Recent developments in feed enzyme technologies has made it possible to incorporate many exogenous enzymes such as phytase, amylase, protease and α-galactosidase into plant-based diet either to improve digestibility of nutrients or to reduce the antinutritive factors (Cao *et al.,* 2008). Supplementation of 10% *Gliricidia* leaf meal with commercial enzymes (Roxazyme and Maxigrain) improved its utilization in catfish, *Clarias gariepinus* diet (Olopade *et al*., 2015). Many other enzymes such as xylanases, β-glucanases, amylase, and protease enzymes used in various types of animal diets have been shown to have a role in improving the quality of plant ingredients fed to animals (Hesselman and Aman, 1986). Bairagi *et al.* (2004) evaluated the nutritive value of *Leucaena leucocephala* leaf meal inoculated with fish intestinal bacteria, *Bacillus subtilis* and *Bacillus circulans* in diets for *Labeo rohita.* They observed considerable reduction in mimosine, tannin and crude fibre contents and increase in crude protein, total free amino acids and fatty acids of leucaena consequent of treatment and reported that it was possible to incorporate leucaena up to 40% level of inclusion in carp diets when enzyme-producing fish intestinal bacteria was added.

Plant Breeding

Another effective strategy to reduce the effects of antinutrients in fish is to utilize improved cultivars of plants which have reduced antinutrient as a result of breeding (Le *et al*., 2016). This strategy has been used to eliminate or substantially reduced several anti-nutritional factors in feed peas including tannins and anti-trypsins (Castell *et al.*, 1996). Improved strains of cassava containing low levels of hydrogen cyanide have been developed over the years by many researchers (Lukuyu *et al*., 2014; Oresegun *et al.,* 2016).

Leaf protein concentrates (LPC)

Another initiative geared towards solving the problems associated with the use of leaf materials holistically is the extraction of protein from plant leaves for aquafeed

production. Such extracted proteins known as concentrates have been shown to have competitive nutritional value in terms of protein quality and amino acid profile relative to the conventional fish and soybean meals with a possible exception of methionine which could be supplemented with synthetic methionine (Table 2) (Akeson and Stahmann, 1966; Oresegun *et al*., 2016). Kinsella (1970) reported that the biological value of LPC is lower than that of whole egg but similar to that of milk and higher than those of casein, beef and soybean. The crude protein content of LPC usually ranges between 40-70% (on dry matter basis) depending on the extraction process and the source of the leaf extracted with appreciable quantities of β-carotene, Vitamin E and minerals (Oke, 1973; Teo *et al*., 2010; Chiesa and Gnansounou, 2011). There are documented evidences that the process of leaf fractionation to produce LPC reduces or totally removes antinutrients such as tannins, hydrocyanic acid, oxalic acids among others in leaf meal (Dewanji *et al*., 1997). Another advantage of LPC is that the fibre fraction in leaf meal has been remarkably reduced (Modesti *et al*., 2007) making it a desirable ingredient in monogastric nutrition (Olvera-Novoa *et al*., 1990; Agbede and Aletor, 2003) whereas the fibrous fraction containing residual protein is quite relevant in ruminant nutrition while the deproteinised juice is often used as fertilizer (Fiorentini and Galoppini, 1983).

The procedure (Fig. 1) for the extraction of leaf proteins has been extensively detailed in the review of Chiesa and Gnansounou (2011) and the work of Coldebella *et al*. (2013). This involves tissue disruption by mechanical means; precipitation of protein using ammonium, sodium or potassium salts, alcohol or acetone, heat, isoelectric point induction or more recently membrane filtration; after which protein is then concentrated by heating or centrifuging. Addition of sodium metabisulfite (an antioxidant) has been experimented with positive result to avoid possible reactions in which the indigestible tannin-protein complexes are formed during protein extraction (Teo *et al*., 2010).

According to Edwards *et al*. (1975) the coagulated proteins can also be fractionated into:

- chloroplastic proteins at 60°C for 20 seconds. Chloroplastic proteins originated from the chloroplasts and often referred to as green proteins because of their association with chlorophyll, carotenoids and lipids which also impart a typical odour and taste.
- cytoplasmic proteins at 80°C for 2minutes, cytoplasmic proteins originates in the cytoplasm, soluble in water and possess a white or yellowish colour.

Coldebella *et al*. (2013) evaluated protein extraction methods for the purpose of making protein concentrates from fresh and dried cassava leaves and recommended precipitating proteins at their isoelectric points and auto-coagulation through fermentation for efficient extraction and industrial application.

Progress made in research relating to LPC production has proven that it is possible to increase the level of leaf meal incorporated as protein ingredients into aquafeed (especially those of herbivores) when they are processed into concentrates. Ngugi *et al*. (2017) demonstrated the suitability of leaf protein from amaranth, a leafy vegetable, in the diet for Nile tilapia, *O. niloticus*. They reported that 80% of fish meal was replaced with the leaf concentrate from amaranth without a growth compromise. Olvera-Novoa *et al*. (1990)

Table 2: Amino acid (g 100g-1 protein) composition of some selected leaf meals

Leaf meal	Arg	His	Leu	Iso	Lys	Met	Cys	Try	Thr	Phe	Tyr	Val	Source
Leucaena leucocephala	4.5	1.5	6.1	6.5	4.8	0.4	0.4	-	3.7	4.4	3.5	4.0	Hasan *et al*. (1997)
Leucaena leucocephala LPC	5.5	2.1	9.1	5.0	6.0	2.3	1.0	1.9	4.6	5.8	4.2	5.8	Agbede and Aletor (2004)
Moringa spp	6.2	3.0	8.7	4.5	5.6	2.0	1.4	2.1	4.7	6.2	3.9	5.7	Makkar and Becker (1996)
Gliricidia spp LPC	6.3	2.5	9.3	5.1	6.6	2.1	1.6	1.9	5.1	6.2	4.8	6.2	Agbede and Aletor (2004)
Trichanthera gigantea	4.9	2.2	7.2	4.1	4.3	1.5	1.4	1.0	4.3	4.6	3.3	5.0	Leterme *et al*. (2005)
Carica papaya	7.2	4.6	8.3	4.9	6.2	0.1	0.0	0.0	5.7	5.7	3.5	3.9	Ganzon-Naret (2015)
Morus alba	5.3	2.1	8.2	4.3	5.7	1.6	1.3	1.1	4.6	5.2	3.4	5.4	Leterme *et al*. (2005)
Ipomoea batata	5.2	2.0	8.8	4.2	4.1	1.6	3.2	-	5.2	6.9	4.0	5.7	An *et al*. (2004)
Azolla pinnata	5.4	-	7.7	4.4	4.6	1.6	0.8	1.8	4.1	4.7	3.2	5.5	Alalade and Iyayi (2006)
Eichhornia crassipes	5.2	2.2	8.3	4.7	5.7	1.4	-	1.0	4.3	5.4	3.4	5.6	Sotolu (2013)
Eichhornia crassipes LPC	3.0	1.9	7.2	4.3	5.3	0.3	11.6	-	4.3	4.7	3.0	0.3	Taylor and Robbins (1968)
Spirodela polyrrhiza	5.7	2.8	9.1	6.0	4.9	Traces	-	1.3	4.1	4.9	4.6	5.8	Bytniewska and Maciejewska-Potapczyk (1980)
Elodea canadensis	8.4	4.5	9.5	4.2	8.3	Traces	-	1.5	3.4	6.1	3.4	5.6	Bytniewska and Maciejewska-Potapczyk (1980)
Riccia fluitans	5.3	3.4	9.8	3.5	6.1	Traces	-	1.6	4.6	6.1	3.1	6.6	Bytniewska and Maciejewska-Potapczyk (1980)
Manihot esculenta	5.3	2.3	8.2	4.5	5.9	1.9	1.4	2.0	4.4	5.4	-	5.6	Ravindran (1991)
Manihot esculenta (LPC)	6.0	2.6	9.6	5.5	6.7	2.5	1.3	2.4	4.9	6.3	4.7	6.2	Fasuyi and Aletor (2005)
Amaranthus hybridus	5.2	1.8	7.8	4.0	6.1	0.3	0.4	-	5.0	4.6	3.6	4.8	Andini *et al*. (2013)
Telfairia occidentalis	5.0	2.1	6.4	4.0	5.2	1.5	0.8	-	3.4	3.6	2.9	4.3	Agbede *et al*. (2012)
Soybean Meal	7.0	2.6	8.0	4.9	6.5	1.4	-	-	4.3	5.4	3.9	4.8	Sotolu (2013)
Fish meal	5.8	2.4	7.5	4.5	7.7	2.9	1.0	1.2	4.3	3.9	3.1	5.4	FAO (2001)

Arg - Arginine, His – Histidine, Leu- Leucine, Iso – Isoleucine, Lys – Lysine, Met – Methionine, Cys – Cystine, Try – Tryptophan, Phe – Phenylalanine, Tyr – Tyrosine, Val - Valine

Source: OECD-FAO Agricultural Outlook 2014-2023

replaced fish meal protein with varying levels (15, 25, 35, 45, 55%) of chloroplastic and cytoplasmic alfalfa LPC in the diet for *Oreochromis mossambicus*. They reported a significantly higher performance relative to the fish meal-based control with the cytoplasmic LPC treatment up to 35% level; beyond which performance was depressed. This is not surprising as Henry *et al.* (1965) and Hernandez *et al.* (1997) established that the chloroplastic protein have lower biological value relative to their cytoplasmic counterpart as a result of association of the former with indigestible components. Levels up to 25% of cassava leaf protein concentrate was included in Nile tilapia diets during sex reversal phase,

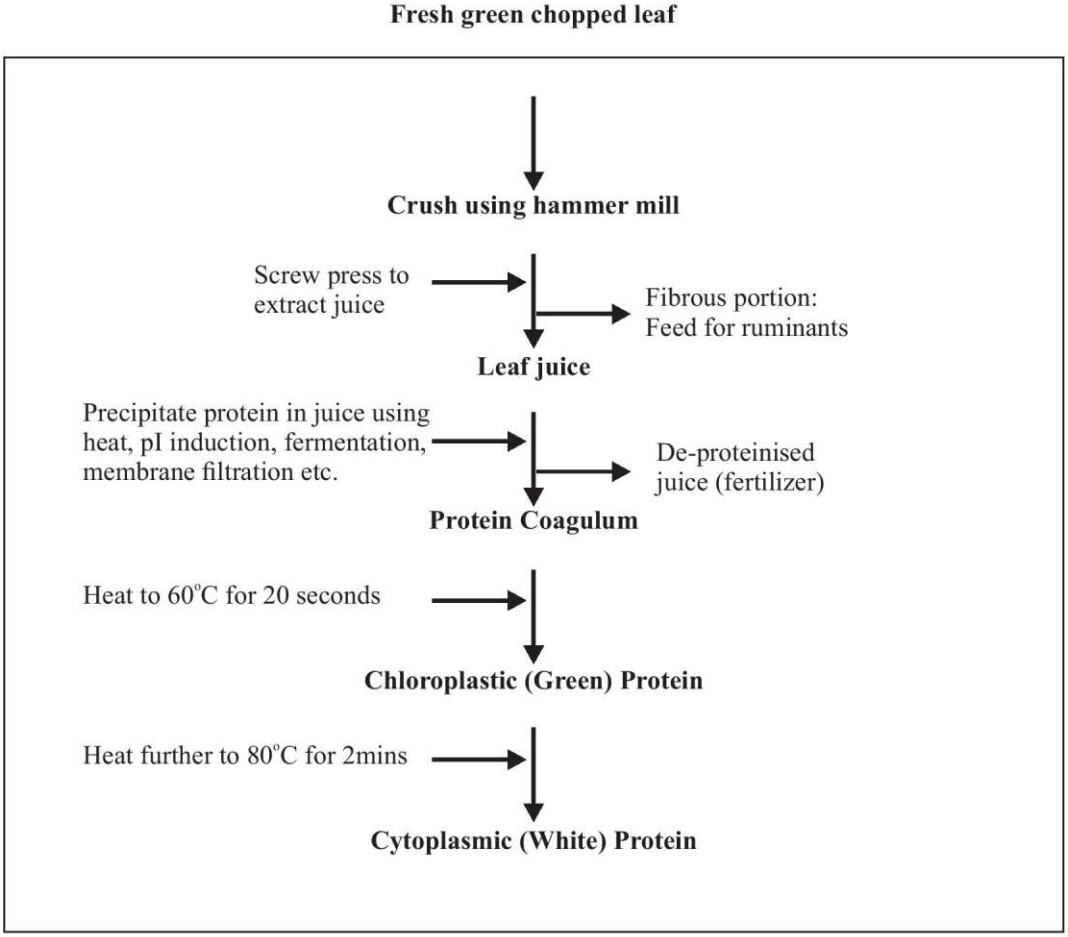

Fig. 1: Preparation of fractionated leaf protein concentrate. Adopted from Fellows (1987).

at the age of 7 days, without any deleterious effects on performance and survival (Bohnenberger *et al*., 2010). Sheeno and Sahu (2006) evaluated Azolla protein concentrate mixed with dried spirogyra powder at 4:1 ratio as a substitute for fish meal and concluded that as much as 16.25% of the plant mixture can be incorporated in the diet of *Labeo rohita* fry. In poultry nutrition, Agbede and Aletor (2003) showed that *Gliricidia* leaf protein concentrate could replace up to 25% fish meal protein, in broiler chick without adverse effect on weight gain, carcass characteristics and health. Ameenuddin *et al.* (1983) had earlier reported an excellent growth and feed efficiency when 40% of low saponin alfalfa protein concentrate was fed to broiler chicks.

CONCLUSION

Apparently, there are numerous unutilized or underutilized plant materials which have global distribution and are not directly consumed by humans; these could represent a cheap source of local feed materials for animal feed industry and thus potentially reduce or eliminate the need to import feed and/or feed ingredients, save foreign exchange and reduce cost; thereby making aquaculture production more economically viable and sustainable. It is gratifying to note that major fish spp responsible for aquaculture growth are those that feed low in the trophic level such as carps and tilapias (FAO, 2012; Ling *et al*., 2015) which have the ability to better utilize plant protein unlike the carnivores. Although, not a few researches on utilization of leaf meals in fish diet have been published; there is still need to intensify efforts in order to provide information on the potentiality of many other available terrestrial and aquatic plant species in aquafeed industry either as protein or energy ingredients.

In the near future, LPC produced from leaf materials, especially those having high protein and dry matter content, good protein extractability when freshly cut and good re-growth potential, will be increasingly relevant in fish feed industry. However, there are some other issues associated with the production of LPC which must be addressed as a matter of future investigation. For instance, most of the work done on LPC utilizes fresh leaf material; this might pose problems relating to storage, transportation and availability during off-season periods. Protocols that will ensure long term storage of the leaves, especially those that are crop by-products or residues, without negatively impacting on their nutritional composition needs to be developed. There is also need for further research on the economic feasibility of extracting protein from dried/stored leaves relative to fresh ones. The problem of high energy cost associated with protein production also needs to be addressed. Oke (1973) had earlier highlighted high energy cost as the major reason for poor adoption of protein production from leaf resource especially in the developing countries. Development of a simple technology for LPC production at commercial scale using a multi-disciplinary research approach might help in this regard. The potential of these abundant and cheap resources should not be overlooked as this may be the key factor for the sustenance of aquafeed industry in future.

REFERENCES

Adeduntan, S. A. and A. S. Oyerinde, 2010. Evaluation of nutritional and anti-nutritional characteristics of Obeche (*Triplochition scleroxylon*) and several Mulberry (*Morus alba*) leaves. *African J. Biochem. Res.*, **4**(7): 175-178.

Adewolu, M. A., 2008. Potentials of sweet potato (*Ipomoea batatas*).Leafmeal as dietary ingredient for *Tilapia zilli* fingerlings. *Pakistan J. Nut.*, **7**(3): 444-449.

Adewolu, M. A. and A. A. Adamson, 2011. *Amaranthus spinosus* leaf meal as a dietary protein source in catfish *Clarias gariepinus* fingerlings. *Int. J. Zool. Resour.,* **7:** 128-138.

Agbede, J. O.,2006.Characterisation of the leaf meals, protein concentrates and residues from some tropical leguminous plants.*J. Sci. Food Agric.,* **86:** 1292–1297

Agbede, J. O., S. A. Adeyeye and M. Adegbenro, 2012.Nutritional, functional property and bioactive components of the leaf products from edible vegetables, *Revista científica. UDO Agrícola* **12**(3): 741-748.

Agbede, J. O. and V. A. Aletor, 2003.Comparative evaluation of weaning foods from *Gliricidia* and *Leucaena* leaf protein concentrates and some commercial brands in Nigeria.*J. Sci. Food Agricult.,* **84:** 21-30.

Agbede, J. O. and V. A. Aletor, 2004. Chemical characterization and protein quality evaluation of leaf protein concentrates from *Glyricidia sepium* and *Leucaena leucocephala. Int. J. Food Sci. Technol.,* **39:** 253–261.

Akeson, W. R. and M. A. Stahmann, 1966. Leaf protein concentrates: A comparison of protein production per acre of forage with that from seed and animal crops. *Econ. Bot.,* **20:**244-250.

Akinyemi, A. F. and I. B. Kayode, 2012. Nutritional Composition of Plant Materials Consumed by Baboon (*Papio Anubis*) and Tantalus Monkeys (*Chlorocebus tantalus*) in Yankari Game Reserve, Nigeria. *J. Primatol.,* **1:**105.

Akwaowo, E. U., B. A. Ndon Bassey and E. E. Etuk, 2000.Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook f.). *Food Chem.,* **70:**235–240.

Alalade, O.A. and E. A. Iyayi, 2006. Chemical composition and feeding value of Azolla (*Azolla pinnata*) meal for egg-type eggs. *Int. J. Poult. Sci.*, **5:**137-141.

Alegbeleye, W.O., S. O. Obasa, O. O. Olude, T. Moronkeji, and I. Abdulraheem, 2012b. Growth performance and nutrient utilization of African Mud Catfish (*Clarias gariepinus*) fingerlings fed different levels of fermented Pigeon Pea (*Cajanus cajan*) meal. Israeli J. Aquacult. - *Bamidgeh*, **64** :731-739.

Aletor, O., A. A. Oshodi and K. Ipinmoroti, 2002. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. *Food Chem.*, **78:** 63–68.

Almazan, A. M., and F. Begum, 1996. Nutrients and anti-nutrient in peanut greens. *J. Food Comp. Anal.,* **9**(43):375-383.

Al-Shorgani, N. K. N., M. S. Kalil, and W. M. W. Yusoff, 2012. Biobutanol production from rice bran and de-oiled rice bran by *Clostridium saccharoperbutylacetonicum* N_{1-4}, *Biopro. Biosyst. Eng.,* **35**(5): 817-826.

Amata, I. A. and L. Bratte, 2008. The effect of partial replacement of soybean meal with gliricidia leaf meal on the performance and organ weights of weaner rabbits in the tropics. *Asian J. Anim. Vet. Adv.*, **3:** 169-173.

Ameenuddin, S., H. R., Bird, D. J. Pringle and M. L. Sunde, 1983, Studies on the utilization of leaf protein concentrates as a protein source in poultry nutrition, *Poultry Sci.*, **62**:505.

Amisah, S., M. A. Oteng and J. K. Ofori, 2009.Growth performance of the African catfish, *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal. *J. Applied Sci.Env. Manag.,* **13:**21-26.

An, L. V., 2004. Sweet potato leaves for growing pigs. Biomass yield, digestion and nutritive value. Doctor's thesis, *Swedish University of Agriculture Sciences*, pp: 49.

Andini, R., S. Yoshida and R. Ohsawa, 2013.Variation in protein content and amino acids in the leaves of grain, vegetable and weedy types of amaranths. *Agron.*, **3**(2): 391–403.

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

Aye, P. A. and M. K. Adegun, 2013. Chemical composition and some functional properties of moringa, *leucaena* and *gliricidia* leaf meals. *Agric. Biol. J. N. Am.* **4**(1): 71-77.

Ayssiwede S. B., A. Dieng,H. Bello, C. A. A. M Chrysostome, M. B. Hane, A. Mankor, M. Dahouda, M. R. Houinato, J. L. Hornick and A. Missohou, 2011. Effects of *Moringa oleifera* (Lam.) leaves meal incorporation in diets on growth performance, carcass characteristics and economic results of growing indigenous Senegal chickens. *Pakistan . Nut.,* **10**(2):1132-1145.

Bach Knudsen, K. E., 2001. The nutritional significance of dietary fibre analysis. *Anim. Feed Sci. Technol.*, **90**: 3-20.

Bairagi, A., K. S. Ghosh, S. K. Sen, and A. K. Ray, 2002. Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu, *Labeo rohita* (Ham.) fingerlings afterfermentation with a fish intestinal bacterium. *Biores. Technol.*, **85:** 17-24.

Bairagi, A., K. S. Ghosh, S. K Sen and A. K. Ray, 2004. Evaluation of nutritive value of *Leucaena leucocephala* leaf meal inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. *Aquacult. Res.*, **35:** 436-446.

Bohnenberger, L., S. D. Gomes, S. R. M. Coelho andW. R. Boscolo, 2010. Concentrado proteico de folhas de mandioca na alimentação de tilápias-do-nilo na fase de reversão sexual. *Revista Brasileira de Zootecnia*, Viçosa-MG, v.39, n.6, p.1169-1174.

Boyd, C.E.,1968. Fresh water plants: A potential source of proteins. *Econ. Bot.,* **22:** 359-368.

Bradbury, J. H., and I. C. Denton, 2011. Mild methods of processing cassava leaves to remove cyanogens and conserve key nutrients. *Food Chem*., **127:** 1755–1759.

Bytniewska, K. and W. Potapczyk, 1981. Growth and contents of total and readily soluble protein in aseptic culture of *Spirodela polyrrhiza* (L.) Schleid. *Acta Physiologiae Plantarum*., 3.

Cao, L., Y. Yang, W. M. Wang, A. Yakupitiyage, D. R. Yuan and J. S. Diana, 2008. Effect of pretreatment with microbial phytase on phosphorus utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquacult. Nut.,* **14:**99–109.

Castell, A.G., W. Guenter andF. A. Igbasan, 1996. Nutritive value of peas for nonruminant diets. *Anim. Feed Sci. Technol.,* **60:** 209–227.

Coldebella, P. F., S. D. Gomes, J. A. Evarini, M. P. Cereda, S. R. M. Coelho and A. Coldebella, 2013. Evaluation of protein extraction methods to obtain protein concentrate from cassava leaf. Eng. Agric. **33**:6.

Checke, P. R. and L. R. Shull, 1985.Natural toxicants in feeds and poisonous plants. Westport, CT: AVI Publishing Co., Inc.

Chiesa, S. and E. Gnansounou, 2011. Protein extraction from biomass in a bioethanol refinery-possible dietary applications: Use as animal feed and potential extension to human consumption. *Biores. Technol.*, **102**(2): 427–436.

Chikwendu, J.N., A. C. Igbatim and I. C. Obizoba,2014.Chemical composition of processed cowpea tender leaves and husks. *Int. J. Sci. Res. Publ.,* **4:**1–5.

Chitra, P. and A. Balasubramanian, 2016. A study on the chemical composition and nutritive value of Albizia tree leaf as livestock feed. International Journal of Science, Environment and Technology, Vol. 5, No 6, 2016, 4638 – 4642

Culley, D. D., E Rejmankova,J.Kevt and J.B. Fry, 1981. Production of chemical quality and use of duckweeds (Lemnaceae) in aquaculture, waste management and animal feeds.*J. World Maricult. Soc.,* **12**(2): 27-49.

Dewanji, A., S. Chanda, L. Si, S. Barik and S. Matai 1997. Extractability and nutritional value of leaf protein from tropical aquatic plants. *Plant Foods for Human Nutrition* **50**: 349-357

Edwards, R. H., R. E. Miller, D. D. Fremery, B. E. Knuckles, E. M. Bickoff and G. O. Kohler, 1975. Pilot plant production of an edible white fraction leaf protein concentrate from alfalfa. *J Agricult. Food Chem.,* **23**(4).

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

Essa, M. A., 1997. Utilization of some aquatic plants in diets for Nile tilapia, *Oreochromis niloticus*, fingerlings. Egypt. *J. Aquat. Biol. Fish.,* **12**: 19–34.

FAO, 1992. Legume trees and other fodder trees as protein sources for livestock.In: Proceedings of the FAO Expert Consultation held at the Malaysian Agricultural Research and Development Institute (MARDI) in Kuala Lumpur, Malaysia, 14–18 October 1991 (Eds. A. Speedy and P. Pugliese). FAO, Rome, 339pp.

FAO, 2001. Windsor M. L. Fish Meal. Torry Advisory Note No. 49.Torry Research Station. FAO Corporate Document Repository (http://www.fao.org/wairdocs/tan/x5926e/x5926e01.htm).

FAO, 2012.The State of World Fisheries and Aquaculture 2012. Rome. 209 pp.

FAO, 2016.The State of World Fisheries and Aquaculture. Contributing to Food Security and Nutrition for All. FAO, Rome, 200 p.

FAO, 2017. FAO Aquaculture Newsletter. No. 56 (April). Rome.

Fasuyi, A. O., 2006. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. *Afr. J. Biotechnol.*, **5:** 49-53.

Fasuyi, A. O., F. A. S. Dairo and A. O. Adeniji, 2008. Tropical vegetable (*Amaranthus cruentus*) leaf meal as alternative protein supplement in broiler starter diets: Bionutritional evaluation. *J. Cent. Eur. Agr.,* **9**(1): 23-34.

Fawole, F. J., N. P. Sahu, A. K. Paland W.S. Lakra, 2013. Evaluation of antioxidant and antimicrobial properties of selected Indian medicinal plants. *Int. J. Med. Arom. Plants*, **3**(1): 69-77.

Fellows, P., 1987. Village-scale leaf fractionation in Ghana. *Trop. Sci.*, **27:**77–84.

Fiorentini, R. and C. Galoppini, 1983. Plant Food. *Hum. Nutr.,* **32:** 335.

Firmani, U., Y. Cahyoko, and Mustikoweni, 2015. Utilization of autoclaved and fermented sesame (*Sesamum indicum* L.) seed meal in diets for Til-aqua natural male tilapia. *Aquacult. Indones.,* **15**(2): 69-72.

Francis, G., Z. Kerem, H. P. S. Makkar and K. Becker, 2002. The biological action of saponins in animal systems: A review. *British J. Nut.,* **88:**587–605.

Francis, G., H. P. S. Makkar, and K. Becker, 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **119:** 197-227.

Gabriel, U. U., O. A. Akinrotimi, D. O. Bekibele, D. N. Onunkwo and P. E. Anyanwu, 2007. Locally produced fish feed, potentials for aquaculture development in sub-Saharan African. *J. Agricult. Res.,* **2:** 287-295.

Garduno-Lugo, M. and M. A. Olvera-Novoa, 2008. Potential of the use of peanut (*Arachis hypogaea*) leaf meal as a partial replacement for fish meal in diets for Nile tilapia (*Oreochromis niloticus* L.). *Aquacult. Res.*, **39:** 1299–1306.

Gangadhar, B., N. Sridhar, S. Saurabh, C. H. Raghavendra, K. P. Hemaprasanth, M.R. Raghunath and P. Jayasankar. 2015. Effect of azolla-incorporated diets on the growth and survival of *Labeo fimbriatus* during fry-to-fingerling rearing. *Cogent Food & Agriculture*, **1**: 1055539.

Ganzon-Naret, E. S., 2015.Effects of incorporated swamp cabbage (*Ipomea aquatica*) and papaya (*Carica papaya*) leaf meals at different dietary levels in order to replace fish meal protein in practical diets for sea bass (*Lates calcarifer*). *ABAH Bioflux,* **7**(1): 93-102.

Gonçalves, A., P. Goufo, A. Barros, R. Domínguez-Perles, H. Trindade, E. A. S Rosa, L. Ferreira and M. Rodrigues, 2016. Cowpea (*Vigna unguiculata* L. Walp) a renewed multipurpose crop for a more sustainable agri-food system. Nutritional advantages and constraints. *J. Sci. Food Agricul.*, **96:** 2941–2951.

Habib, G., Habib, N. A., Khan, A., Sultan, M. and Ali, N. A. 2016. Nutritive value of commontreeleaves for livestock in the semi-arid and arid rangelands of Northern Pak. *Livest. Sci.,* **184:**64-70.

Hall, G. M., 2015. Impact of Climate Change on Aquaculture: The Need for Alternative Feed Components. *Turk. J. Fish. Aquat. Sc.,* **15:** 569-574.

Hardy, R. W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquacult. Res.*, **41:** 770–776.

Hasan, M. R., D. J. Macintosh andK. Jauncey, 1997. Evaluation of some plant ingredients as dietary protein sources for common carp (*Cyprinus carpio*) fry. *Aquaculture*, **151:**55-70.

Hasan, M. R., M. Moniruzzaman and A. M. Farooque, 1990. Evaluation of leucaena and water hyacinth leaf meal as dietary protein sources of the fry of Indian major carp, *Labeo rohita* (Hamilton), In: Proceedings of the Second Asian Fisheries Forum (Eds. R. Hirano and I. Hanyu), Asian Fisheries Society, Manila, Philippines, pp. 275-278.

Henry, K. M. andJ. E. Ford, 1965. Nutritive value of leaf protein concentrates determined in biological tests with rats and by microbiological methods. *J. Sci. Food Agricult.*, **16**: 425-432.

Hernández, T., C. Martínez, A. Hernández and G. Urbano, 1997. Protein quality of alfalfa protein concentrates obtained by freezing. *J. Agricult. Food Chem.,* **45**(3): 797–802.

Hesselman, K. and P. Aman, 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. *Anim. Feed Sci. Technol.*, **15**: 83-93.

Heuze, V., G. Tran, P. Noziere, D. Bastianelli and F. Lebas, 2015. Cowpea (*Vigna unguiculata*) forage. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. *https://www.feedipedia.org/node/233 Last updated on October 20, 2015, 16:20.*

Igbinosun, J. E. and S. O. Talabi, 1982. Studies on the nutrition of brackish water catfish: *Chrysichthys nigrodigitaius*. Paper presented at the 2nd Conference of Fisheries Society of Nigeria, Calabar, 24-27 January, 1982, Technical Paper No. 6.

Iheukwumere, F. C., E. C. Ndubusi, E. A. Mazi and M. U. Onyekwere, 2007. Growth, Blood chemistry and carcass yield of broilers fed cassava leaf meal (*Manihot esculenta* Crantz) Inter. *J. Poult. Sci.,* **6**(4): 255-260.

Imungi, J. K. and N. N. Potter, 1983. Nutrient contents of raw and cooked cowpea leaves. *J. Food Sci.,* **48:** 1252–1254.

Islam, K. M. S., 2002. Feasibility of duckweed as poultry feed - a review. *Indian J. Anim. Sci.*, **72** (6): 486-491.

Jaarsveld, P. V., M. Faber, I. V. Heerden, F. Wenhold, W. J. V. D. Rensburg and W. V. Averbeke , 2014. Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. *J. Food Compos. Anal.,* **33:**77–84.

Kambashi, B., P. Picron, C. Boudry, A. Théwis, H. Kiatokoand J. Bindelle, 2014. Nutritive value of tropical forage plants fed to pigs in the Western provinces of the Democratic Republic of the Congo. *Anim. Feed Sci. Tech.*, **191**: 47–56.

Kinsella, J. E., 1970. Evaluation of plant leaf protein as a source of food protein. *Chem. Ind.,* **17:** 550-554.

Kumar, V., A. K. Sinha, H. P. S. Makkar, G. De Boeck and K. Becker, 2011. Phytate and phytase in fish nutrition. *J. Ani. Phy.Ani.Nutr.*doi: 10.1111/j.1439-0396.2011.01169.x

Le, D. T., H. D. Chuand N. Q. Le, 2016.Improving nutritional quality of plant proteins through genetic engineering. *Curr. Genom.*, **17**(3): 220–229.

Leng, R. A., J. H. Stambolie and R. Bell, 1995. Duckweed-a potential high-protein feed resource for domestic animals and fish. *Livest.Res. Rural Dev.*, **7**(1): 36.

Leterme P, A. M. Londoño, F. Estrada, W. B. Souffrant and A. Buldgen, 2005. Chemical composition, nutritive value and voluntary intake of tropical tree foliage and cocoyam in pigs. *J. Sci. Food Agric.,* **85:** 1725-1732.

Ling, C., R. L. Naylor, P. Hendriksson, D. Leadbitter, M. Metian, M. Troell and Z. Wenbo, 2015. China's aquaculture and the world's wild fisheries. *Science*, **347**(6218): 133-135.

Little, E. C. S., 1979. Handbook of utilization of aquatic plants. *FAO Fish.Tech.Pap.,*187: 176. Lukuyu, B., I. Okike, A. Duncan, M. Beveridge and M. Blümmel, 2014. Use of cassava in livestock and aquaculture feeding programs.ILRI Discussion Paper 25. Nairobi, Kenya: International Livestock Research Institute.

Makkar, H. P. S., 1993. Antinutritional factors in foods for livestock. In: Animal Production in Developing Countries (Eds. M. Gill, E. Owen, G. E. Pollot and T. L. J. Lawrence), Occasional publication No. 16. *Brit. Soc. Anim. Prod.,* pp. 69–85.

Makkar, H. P. S. and K. Becker, 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oliefera* leaves. *Anim. Feed Sci. Tech.,* **63:** 211-228.

Mako, A. A., O. J. Babayemi and A. O. Akinsoyinu, 2011. An evaluation of nutritive value of water hyacinth (*Eichhornia crassipes* Mart. Solms-Laubach) harvested from different water sources as animal feed. *Livest.Res. Rural Dev.*, **23** (5).

Maundu, P., G. W. Ngugiand C. H. S.Kabuye, 1999. Traditional food plants of Kenya. National Museums of Kenya, Nairobi, Kenya.

Modesti, C. F., A. D. Correa, E. D. Oliveira, C. P. M. Abreu, C. D. Santos, 2007. Caracterização de concentrado protéico de folhas de mandioca obtido por precipitação com calor e ácido.*Ciência e Tecnologia de Alimentos*, Campinas, v.27, n.3, p.464-469.

Mohapatra, S. B. 2015. Utilization of water hyacinth (*Eichhornia crassipes*) meal as partial fish protein replacement in the diet of *Cyprinus carpio* fry. *Eur. J. Exp. Biol.*, **5**(5):31-36.

Mondal, T. K. and A. K. Ray, 1999. The nutritive value of *Acacia auriculiformis* leaf meal in compound diets for *Labeo rohita* fingerlings. In: The Fourth Indian Fisheries Forum Proceedings, Asian Fisheries Society, Indian Branch, Mangalore, India (Ed. J. M. Mohan), pp. 295-298.

Morgan, N. K. and M. Choct, 2016. Cassava: Nutrient composition and nutritive value in poultry diets. Anim. Nut., **2**(4): 253-261.

Naegel, L. C. A., 1997. Azolla meal as a supplemental feed ingredient for tilapias. In: 4th Intl. Symp. on Tilapia in Aquaculture Proc. (Ed. Fitzimmons, K.) Orlando, FL, USA, 9–11 November 1997, pp. 20–30.

Naylor, R.L., R.W. Hardy, D. P. Bureau, A. Chiu, M. Elliot, A. P. Farrell, I. Forster, D. M. Gatlin, R. J. Goldburg, K. Hua and P. D. Nichols, 2009. Feeding aquaculture in an era of finite resources.*Pro.Nat. Acad. Sci., USA*, **106** (36):15103–15110.

Ncha, O. S., P. B. Michael,U. O. Nnabuchi and E. Alex, 2015. Effect of diets with Moringa leaf meal on growth, carcass composition and haematology of *Clarias gariepinus.Int. J. Fish. Aquat. Stud.,* **3**(2):397-401.

Ngugi, C.C., E. Oyoo-Okoth, J. O. Manyala, K. Fitzsimmons, and A. Kimotho, 2017. Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds. *Aquacult. Rep*., **5:** 62–69.

Nieves, P. and R. Barro, 1996.Evaluation of local ingredients (fish, shrimp, snail, copra, leaf meals and rice bran) for feeding Nile tilapia (*Oreochromis niloticus*) fingerlings. In: Proceedings of the national workshop on fish nutrition and feeds, 1–2 June 1994, Iloilo, Philippines (Eds. C. B. Santiago, R. M. Coloso, O. M. Millamena, and I. G. Borlongan). Iloilo, Philippines: Southeast Asian Fisheries Development Centre (SEAFDEC).

Nisha, N. S. and B. Geetha, 2017.Effect of partial replacement of fishmeal with aquaticweed *Pistia stratiotes* meal on growth, biochemical composition, haematological parameters and digestive enzymes in Indian major carp *Labeo rohita* (Hamilton, 1822). *Int. J. Fish.Aquat. Stud.,* **5**(2): 527-532.

Nkosi, B. D., R. Meeske, T. Langa, M. D. Motiang, S. Modiba, N. R. Mkhize, and I. B. Groenewald, 2016. Effects of ensiling forage soybean (*Glycine max* (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion of the silage by Damara rams. *Small Ruminant Res.,* **134:**90-96.

Nworgu, F. C. and G. N. Egbunike, 2013. Nutritional potential of *Centrosema pubescens, Mimosa invisa and Pueraria phaseoloides* leaf meals on growth performance responses of broiler chickens". *Am. J. Exp. Agr.,* **3** (3): 506–519.

Oke O. L., 1973. Leaf protein research in Nigeria.A review.*Trop. Sci.,* **15:**139–155.

Olopade, O. A., A. A. Lamidi, and M. O. Ogungbesan, 2015.Effect of *Gliricidia sepium* (Jacq) leaf meal supplemented with enzymes (Roxazyme® G2 and Maxigrain®) on growth performance of *Clarias gariepinus* Burchell, 1822.*Am. J. Exp. Agr.,* **8**(3): 152-158.

Olude, O. O.and O. E. Badmus, 2015. An evaluation of mixture of moringa (*Moringa oleifera*) leaf and kernel meals as partial replacement for fish meal in the diet of *Clarias gariepinus* juveniles. *J. Aquat. Sci.,* **30** (2):347-349.

Olvera-Novoa, M. A., G. S. Campos, G. M. Sabido and C. A. Martınez-Palacios, 1990. The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). *Aquaculture*, **90:** 291–302.

Oresegun, A., O. A. Fagbenro, P. Ilona and E. Bernard, 2016. Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed. *Cogent Food & Agriculture*, **2**: 1147323.

Osman, M. F., E. Omar and A. M. Nour, 1996. The use of leucaena leaf meal in feeding Nile tilapia. *Aquacult.Int.,* **4**: 9–18.

Ozovehe, B. N., 2013. Growth performance, haematological indices and some biochemical enzymes of juveniles *Clarias gariepinus* (Burchell 1822) fed varying levels of *Moringa oleifera* leaf meal diet. *J. Aquacult. Res. Dev.*, **4** (2): 166.

Paranamana, N., K. Radampola, and V. P. Bulugahapitiya, 2015. Nutritional and anti-nutritional contents of alternative plant feed ingredients for fish feed formulation *Indian J. Anim. Sci.,* **85**(2): 212–215.

Priyadharshini, S., S. Bragadeeswaran, K. Prabhu and S. S. Ran, 2011. Antimicrobial and hemolytic activity of seaweed extractsUlvafasciata(Delile 1813) from Mandapam, Southeast coast of India. Asian Pac. J. Trop. Biomed., **1**: S38-S39.

Raja, K. S., F. S. Taip, M. M. Z. Azmi and M. R. I. Shishir, 2017. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. *J. Saudi. Soc. Agricult. Sci.,* https://doi.org/10.1016/j.jssas.2017.04.001.

Ravindran, V., 1991. Preparation of cassava leaf products and their use as animal feed. In: Roots, tubers, plantains and bananas in animal feeding (Eds. D Machin and Solveig Nyvold). FAO Animal Production and Health Paper No **95**: 111-122.

Reyes, O. S. and A. C. Fermin, 2003.Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone *Haliotis asinina* (Linnaeus 1758). *Aquacult.Res.*, **34**: 593–599.

Robaina, L., M. S. Izquierdo and F. J. Moyano,1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. *Aquaculture*, **130**: 219-233.

Riche, M. and P. B. Brown, 1996. Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. *Aquacult.*, **142**:269-282.

Richter, N., S. Perumal and B. Klaus, 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture*, **217**: 599–611.

Saha, A. K. and Ray, A. K., 1998. Cellulase acitivity in rohu fingerlings. *Aquacult. Int.,* **6:**281–291.

Saha, S. and A. K. Ray, 2011. Evaluation of nutritive value of water hyacinth (*Eichhornia crassipes*) leaf meal in compound diets for rohu, *Labeo rohita* (Hamilton, 1822) fingerlings after fermentation with two Bacterial strains isolated from fish gut. *Turkish J. Fish. Aquati. Sci.,* **11:** 199-207.

Sese, B. T., M. Okpeku and I. Patani, 2013. Tropical velvet bean (*Mucuna utilis*) leaf meal as unconventional protein supplement in the diet of broilers. *J. Anim. Sci. Adv.*, **3**(11): 575-583.

Sheeno, T. P. and N. P. Sahu 2006.Use of freshwater aquatic plants as a substitute of fishmeal in the diet of *Labeo rohita* fry. *J. Fish. Aquat. Sci.,* **1**: 126-135.

Shokunbi, O. S., O. A. Anionwu, O. S. Sonuga and G. O. Tayo, 2011. Effect of post-harvest processing on the nutrient and anti-nutrient compositions of *Vernonia amygdalina* leaf. *African J. Biotechnol.*, **10**(53):10980-10985.

Sinha, A. K., V. Kumar, H. P. S. Makkar, G. De Boeck, and K. Becker, 2011. Non-starch polysaccharides and their role in fish nutrition- a review. *Food Chem.,* **127:** 1409-1426.

Sivani, G., Reddy, D. C. and M. Bhaskar, 2013. Effect of Nymphaea meal incorporated diets on growth, feed efficiency and body composition in fingerlings of *Cyprinus carpio* L. *J. Appl. & Nat. Sci.*, **5**(1):5-9.

Sotolu, A. O., 2013. Management and utilization of weed: Water hyacinth (*Eichhornia crassipes*) for improved aquatic resources. *J. Fish. Aquat. Sci.,* **8:**1-8.

Sutriana, A., 2007. The use of cassava leaves as a dietary component for African catfish fry. *J. Ked. Hewan,* **1**(2):59–65.

Stahmann, M. A., 1968. The potential for protein production from green plants. *Econ Bot.*, **22**: 73.

Steinkraus, K. H. 2009. Fermented Foods. *Applied Microbiol.*, 45-53.

Tacon, A. G. J., and M. Metian, 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture,* **285:**146–158.

Talpur, A. D., Ikhwanuddin, M. 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi. Fish Shellfish Immunol.*, **34:** 254-264.

Tan, Y. T., 1970. Comparison and nutritive value of some grasses, plants and aquatic weeds tested as diets. *J. Fish Biol.,* **2:** 253.

Taylor, K. G. and R. C. Robbins, 1968. The amino acid composition of water hyacinth (*Eichhornia crassipes*) and its value as a protein supplement. *Hyacinth Cont. J.* **7:**24–25.

Tekle Esayas Welday, N. P. Sahu and M. Makesh, 2015. Antioxidative and antimicrobial activities of different solvent extracts of *Moringa oleifera*: an *in vitro* evaluation. IJSRP, **5**(5): 255-266

Teo, C. R. P. A., S. H. Prudencio, S. R. M. Coelho and M. S. Teo, 2010. Obtenção e caracterização físico-química de concentrado protéico de folhas de mandioca. *Revista Brasileira de Engenharia Agrícola e Ambiental*, Campina Grande, **14**(9): 993-999.

Vadivel, V. and M. Pugalenthi, 2009. Effect of soaking in sodium bicarbonate solution followed by autoclaving on the nutritional and antinutritional properties of velvet bean seeds. *J. Food Proc. Preserv.*, **33**:60-73.

Vhanalakar, S. A. and D. V. Muley, 2014. Effect of Dietary incorporation of *Gliricidia maculata* leaf meal on growth and feed utilization of *Cirrhinus mrigala* fingerlings. *Global J. Sci. Frontier Res. Biol. Sci.,* **14** (1): 47-49.

Yan X *et al*1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci.Biotechnol.Biochem.,* **63:**605–607.

Yilmaz, E., I. Akyurt and G. Gunal, 2004.Use of duckweed, Lemna minor, as a protein feedstuff in practical diets for common carp, Cyprinus carpio, fry. *Turkish J. Fish. Aquat.Sci.*, **4:** 105-109.

Wee. L., 1991. Use of non-conventional feedstuff of plant origin as fish feeds - is it practical and economically feasible? p. 13-32. In: Fish nutrition research in Asia (Ed. S. S. De Silva) *Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fish. Soc. Spec. Pub. 5:*205 p. Asian Fisheries Society, Manila, Philippines.

World Bank. 2013. Fish to 2030: prospects for fisheries and aquaculture. Agriculture and environmental services discussion paper; no. 3. Washington DC; World Bank Group. http://documents.worldbank.org/curated/en/ 458631468152376668/Fish-to-2030-prospects-for-fisheries-and-aquaculture.