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N Daniel

Assistant Professor,

Tamil Nadu Fisheries

University, Tamil Nadu, India

A review on replacing fish meal in aqua feeds using plant protein sources

N Daniel

Abstract

Until recently, fish meal was the chief protein source in the fish feed for diverse reasons collectively for its high protein content, excellent essential amino acid (EAA) profile, better nutrient digestibility, lack of anti-nutritional factors (ANFs), low price and ease in its availability. However ideal protein source of fish meal for fish feed is now at risk that threatens feed formulators to rely more on this. This example additionally makes feed formulators to look for alternative feedstuffs which can doubtlessly replace fish meal. Plant protein sources are acknowledged as the best source to replace fish meal; but they have contrasting characteristics to those of fish meal due to following attributes: Plant ingredients have ANFs, deficient in certain EAA, low nutrient digestibility, lesser nutrient bio-availability and palatability because of excessive degrees of non-soluble carbohydrates consisting of fibre and starch. These evaluation characters attributed to plant proteins have raised the controversy amongst feed nutritionists that how they can ably replace fish meal. Consistent with available evidences from research findings, it is found possible that plant proteins can replace fish meal either in part or completely when certain dietary recommended conditions are provided that are discussed in the review. Continuing further, the effects of dietary plant proteins on feeding, nutrient utilization and growth performances, protein retention, digestibility and bio-availability of nutrients, variations in biochemical compositions, flesh quality and immunity and stress responses of aquatic animals are individually discussed together with the idea of giving new avenues for future research in the current topic.

Keywords: Bio-availability, diet, digestibility, feed intake, fish meal, growth, immunity, performances, plant proteins, recommended conditions

Introduction

Compared to other animal food-production sectors, aquaculture growth is really worth looking (FAO, 2016) ^[1]. At present, one out of three fishes is coming from aquaculture that is being consumed by human population. It is worth noting that sustainability of aquaculture depends on many factors, including cost effective feed. Feed constitutes around 60 % of total operating cost in the aquaculture; therefore the remarkable growth of aquaculture will be greatly benefited by the development of cheaper aqua feed. The feed formulator's efforts to prepare the feed at lower cost will directly reflect in the economy of fish farmers. Fish meal is one of the principal protein ingredients in the fish diet; it is rich in protein content, properly-balanced EAA profile and excellent nutrient digestibility and deficient in the ANFs. Until recently, fish feed was prepared with fish meal as an important protein ingredient because one would generally agree that fish meal requirement for omnivorous is about 30 to 40% and for carnivorous it is more than 40 %. However, fish meal inclusion levels for both omnivorous and carnivorous fishes have been reducing significantly at present (Hardy, 2010) ^[2] on account of fish meal supply becoming significantly low together with its huge demand and higher prices in the market (Edwards *et al.*, 2004; De-Silva and Hasan, 2007; Hung *et al.*, 2007) ^[3-5]. As an alternative to fish meal, many authors have recommended the plant based protein ingredients specifically regarding the cost as they seem to be cheaper compared to fish meal. But to become a suitable alternative to fish meal, a candidate ingredient ought to own the previously mentioned characteristics which equal fish meal. In this connection, one can disagree for the utilization of plant proteins for the replacement of fish meal in the fish diet based on the following criteria: Plant ingredients have ANFs, are deficient in certain EAA, have less nutrient digestibility, have lesser nutrient bio-availability, and less palatability due to high levels of non-soluble carbohydrates such as fibre and starch.

Correspondence

N Daniel

Assistant Professor,

Tamil Nadu Fisheries

University, Tamil Nadu, India

Many previous reports, therefore, did not recommend replacing fish meal in the diets (De Francesco *et al.*, 2004; Engin *et al.*, 2005; Bonaldo *et al.*, 2011) ^[6-8]. On the other hand, several authors agreed that plant ingredients can be used to replace fish meal in the diet if the animal showed no difference in the overall performances while being fed plant feed (Espe *et al.*, 2007; Hansen *et al.*, 2011; Lund *et al.*, 2011; Yun *et al.*, 2012; Valante *et al.*, 2016; Daniel, 2017) ^[9-14]. Results from the several numerous researches also demonstrated that animals fed with plant proteins did not affect the performance of the animals (Merrifield *et al.*, 2010; Sheikhzadeh *et al.*, 2012; Kpundeh *et al.*, 2015; Guo *et al.*, 2016; Li *et al.*, 2016) ^[15-19].

Shortly fish meal will no longer be a major protein ingredient in the fish diet and it is likely that soon diet free of fish meal will get popularised. Consequently, the development and sustainability of future aquaculture could significantly depend on the identification of new suitable less costly alternative plant protein ingredients that can replace fish meal without compromising the performance of the animals (Gatlin *et al.*, 2007) ^[20]. Although some research findings have revealed the negative consequences of plant protein feeding on animal performances, but several previous reports have also manifested that by implementing of certain following dietary techniques one can feed plant protein diets to the animals sustainably without affecting the animal performances: Those conditions encompass the addition of deficient amino acids (Goda *et al.*, 2007) ^[21], aggregate of different plant sources (Liti *et al.*, 2006) ^[22], application of exogenous enzymes (Jiang *et al.*, 2014) ^[23], adoption of one day plant based and next day fish meal based feed (Nandeeshia *et al.*, 2002) ^[24], supplementation of certain additives (Øverland *et al.*, 2000; Aksnes *et al.*, 2006a; Sarker *et al.*, 2007; Johnson *et al.*, 2015) ^[25-28] and other novel dietary tactics (Lee *et al.*, 2015) ^[29]. The encouraging outcomes from the afore-said previous works inspired many of the researchers to set up work on replacement of fish meal by cheap and alternative plant protein sources. The present paper critically reviewed the various works carried out for the fish meal replacement using plant based protein ingredients in fish and aimed to discuss about the results of plant proteins on fish performances whilst replacing fish meal in the diets. Obviously, the information showed in the present paper would be an impetus for feed formulators to increase the usage of plant protein ingredients for the preparation of aquaculture diets. Simultaneously it will encourage minimising the fish meal usages to ensure the preparation of cost effective diets for the sustainability of fish farmers relying on fish meal for the feed usages.

Effect of plant proteins on feeding, nutrient utilization and growth performances in fish

Previous research has discovered that aquatic animals fed with fish meal depleted diets generally tend to decrease their feed intake and growth performances. The reduction in the feeding and growth with response to higher levels of dietary plant proteins has been reported in several aquatic animals

such as rainbow trout (Gomes *et al.*, 1995; Adelizi *et al.*, 1998; De Francesco *et al.*, 2004; Snyder *et al.*, 2012) ^[30-32], European sea bass (Dias *et al.*, 1997) ^[33], shrimp (Sudaryono *et al.*, 1999) ^[34], turbot (Fournier *et al.*, 2004) ^[35], Atlantic salmon (Berge *et al.*, 1998; Sveier *et al.*, 2001; Espe *et al.*, 2007) ^[36, 37, 9], gilthead sea bream (Gomez-Requeni *et al.*, 2004) ^[38], turbot (Bonaldo *et al.*, 2011) ^[8], black tiger shrimp (Richard *et al.*, 2011) ^[39], eel (Engin *et al.*, 2005) ^[7] and abalone (Bautista-Teruel *et al.*, 2003) ^[40]. Torstensen *et al.*, (2008) ^[41] additionally confirmed that concomitant replacement of fish meal and fish oil with plant proteins and vegetable oils that were fed to Atlantic salmon decreased its feed consumption and growth. Various authors explained the reasons for these causes: the nature of plant proteins having less apparent digestibility coefficient (Gatlin *et al.*, 2007) ^[20], intestinal damage (Yu *et al.*, 2015) ^[42], deficiency of one or more EAAs (Bautista-Teruel *et al.*, 2003) ^[40], less palatability (Torstensen *et al.*, 2008) ^[41] and presence of ANFs (Welker *et al.*, 2016) ^[43]. On the other hand, few authors linked this with the elevated muscle protein degradation (Snyder *et al.*, 2012) ^[32]. There are others who mentioned that the decreased growth rate determined in fish fed diets containing high levels of plant proteins is linked with the modifications in the morphology of their muscle fibres and skeletal muscle and lysosomal proteolysis (Alami-Durante *et al.*, 2010) ^[44]. Contrary to fore-mentioned study reports, it has been substantially established by the findings of following workers that plant proteins can potentially substitute fish meal in the diet of fish without having negative effect on growth or feed intake. But inclusion level of plant ingredients to the diet varies with the species. Lund *et al.*, (2011) ^[11] showed that matrix of organic plant protein concentrates consisting of pea: horse bean: rapeseed can ably replace 44% of the total dietary protein fish meal without causing any negative performances to rainbow trout. Comparable results have also been found in Senegalese sole when fed with blend of plant proteins (soybean meal, peas, corn gluten, and wheat), supporting that the growth performance was not impaired up to 75% in the diet (Valente *et al.*, 2016) ^[13]. Bonaldo *et al.*, (2011) ^[88] supported that mixture of plant protein diets made up of soybean meal, wheat gluten meal and corn gluten meal fed up to 39% levels did not disturb the growth rate and nutrient utilization in turbot. Results from Palmegiano *et al.*, (2008) ^[45] showed that fish meal and fish oil in the diet can be partially replaced with Spirulina meal when integrated with plant oils without having any negative effect to white sturgeon. Hansen *et al.*, (2011) ^[10] discovered that Atlantic cod had the same growth rate with or without the addition of lysine and methionine while fish meal was replaced by 65% with the mixture of plant proteins. Daniel (2016a) ^[46] counselled that water washed neem seed cake may probably replace the fish meal at 5% in common carp and 25% in African catfish respectively without compromising fish growth and nutrient utilization. Table 1 also shows the lists of some studies supporting that fish meal partially replaced by plant protein in fish diets did not affect the animal's performances.

Table 1. A List of some studies supporting that fish meal partially replaced by plant protein in fish diets does not affect the animal's performances

S. No	Species studied	Plant Ingredients used	Supported inclusion level	Remarks	References
1	Rainbow trout	Plant proteins supplemented with lysine	50%	Improved growth performance, feed conversion ratio and survival.	Cheng <i>et al.</i> (2003) [47]
2	European sea bass	Corn gluten meal, wheat gluten, extruded wheat, soybean meal and rapeseed meal.	95%	No adverse effect on somatic growth or nitrogen utilisation.	Kaushik <i>et al.</i> (2004) [48]
3	Gilthead sea bream	Mixture of plant protein sources	75%	Growth performance was not affected.	De Francesco <i>et al.</i> (2007) [49]
4	Atlantic cod	Mix of soybean meal, soy protein concentrate and wheat gluten meal	50%	Growth was hardly affected.	Hansen <i>et al.</i> (2007) [50]
5	Pacific white shrimp	Combination of soybean meal and canola meal	80%	Not affected the growth performances.	Suarez <i>et al.</i> (2009) [51]
6	Cobia	Mixture of plant proteins	94%	No changes in the growth performances compared to fish meal diets.	Salze <i>et al.</i> (2010) [52]
7	Turbot	Mixture of soybean meal, wheat gluten meal and corn gluten meal	52%	Did not reduce the feed intake.	Bonaldo <i>et al.</i> (2011) [8]
8	Rainbow trout	Combination of pea, horse-bean and rapeseed	44%	No negative performances on growth.	Lund <i>et al.</i> (2011) [11]
9	Black tiger shrimp	Mixture of corn gluten meal, rapeseed meal, sorghum and wheat gluten	25%	No adverse effect on shrimp performances.	Richard <i>et al.</i> (2011) [39]
10	Grass carp	Cotton seed meal, sunflower meal and corn meal	75%	No adverse consequence in somatic growth and nitrogen utilization.	Köprücü and Sertel (2012) [53]
11	Hybrid sturgeon	Corn gluten meal	55%	Did not affect the growth and FCR with 30 % of feed price reduction as compared to fish meal diets.	Sicuro <i>et al.</i> (2012) [54]
12	Kuruma shrimp	Mixture of soybean meal and canola meal	50%	No adverse effects on growth, feed utilization, body composition and nutrient utilization.	Bulbul <i>et al.</i> (2013) [55]
13	Senegalese sole	Mixture of plant protein sources with EAAs	75%	No impairments on feed intake, growth performance and protein utilisation.	Cabral <i>et al.</i> (2013) [56]
14	Red drum	Mix of soy protein concentrate and barley protein concentrate	50%	No effect on the growth performance, condition indices and whole-body composition.	Rossi <i>et al.</i> (2013) [57]
15	Senegalese sole	Mixture of soybean meal, soybean protein concentrate and wheat gluten meal	30%	No changes in the growth performances as compared to fish meal diets.	Rodiles <i>et al.</i> (2015) [58]
16	Common carp	Defatted rubber seed meal	50%	No negative effect on the growth and feeding performances.	Suprayudi <i>et al.</i> (2015) [59]
17	Turbot	Fish meal combined with mixture of plant proteins	50 %	Positively affected the growth performance and welfare status.	Bonaldo <i>et al.</i> (2015) [60]
18	Chinese sucker	Mix of fermented soybean meal, corn gluten meal and cottonseed meal with lysine	30%	No adverse effects on growth performance, body composition and digestive enzyme activities.	Yu <i>et al.</i> (2014) [61]
19	Shortfin corvina	Mix of soybean protein concentrate and corn protein concentrate	75%	No compromising effect on growth performance.	Minjarez-Osorio <i>et al.</i> (2016) [62]
20	Senegalese sole	Blend of soybean meal, peas, corn gluten, and wheat	75%	Growth performance was not impaired.	Valente <i>et al.</i> (2016) [13]

In addition to studies represented in the table 1, there are other works also support the replacement of fish meal using plant based ingredients in the fish diet when they added with certain dietary components without much interfering the performances of the animals when certain dietary components are added in the plant diets which include supplementation of crystalline amino acids (Espe *et al.*, 2006) [63], 0.5% methionine, 1.0% lysine, 0.04% phytase and 10% fish soluble (Bulbul *et al.*, 2015) [64], 5% fish meal, 5% fish soluble and 3% squid hydrolysate (Espe *et al.*, 2007) [9], limiting amino acids such as arginine, histidine and threonine (Goda *et al.*, 2007) [21], multiple EAA and krill meal and water soluble fraction of krill (Zhang *et al.*, 2012) [65], feeding stimulants such as Alanine, serine, inosine-5'-monophosphate and betaine (Papatryphon *et al.*, 2001a) [66], taurine (Johnson *et al.* (2015) [28], duckweed (He *et al.*, 2013) [67], squid meal (Silva *et al.*, 2010) [68], salmon testis meal (Lee *et al.*, 2015) [29],

freeze-dried hydrolysate from squid, scallop, krill, worms, or mussel (Kader *et al.*, 2012; Nagel *et al.*, 2014) [70-71], citric acid (Sarker *et al.* (2007; Zhang *et al.*, 2016) [27, 74], However, few attempts had failed to show positive influence on feeding and growth of fish when fed using plant diets supplemented with certain dietary components which include fish meal (Fontainhas-Fernandes *et al.*, 1999) [69], dry hydrolysate from squid and scallop (Zhou *et al.*, 2016) [72], fish hydrolysate (Aksnes *et al.*, 2006b) [85] and water soluble fraction from marine protein sources (Aksnes *et al.*, 2006a) [26]. Interestingly, *Macrobrachium rosenbergii* fed with diets contain equal proportion of plant and animal proteins gave better growth rate and feed conversion efficiency (Hari and Madhusoodana Kurup, 2003) [73]. Hydroxyproline is needed for the production of glycine, pyruvate, and glucose (Wu *et al.*, 2011) [75]. Previous researches have suggested that plant protein sources have

lesser levels of hydroxyproline (Li *et al.*, 2011) [76], taurine (Yamamoto *et al.*, 1998) [77], and cholesterol (Cheng and Hardy, 2004) [78]. However, Zhang *et al.* (2013) [79] reported that turbot fed plant proteins in supplementation with hydroxyproline showed no differences on the growth performances. Generally, organic acids offer energy for growth (Eisemann and Van Heugten, 2007; Topping and Clifton, 2001) [80, 82], immunity (Jongbloed *et al.*, 2000; Øverland *et al.*, 2000) [81, 25] and gut health (Hamer *et al.*, 2008) [83]. However, Gao *et al.* (2011) [84] found that supplementation of plant protein-based diets with a mixture of sodium formate and butyrate did not improve growth rate or feed utilization of rainbow trout. Cholesterol is required for crustaceans in the diet (Holme *et al.*, 2006) [86]. Fish meal contains high levels of cholesterol; however, in most plant sources cholesterol content is significantly much low (Deng *et al.*, 2010) [87]. It was reported that dietary cholesterol improves the feed intake and growth performances in fish when fed with plant based protein diets (Twibell and Wilson, 2004; Chen, 2006) [88-89]. Yun *et al.* (2012) [12] showed that dietary supplementation of cholesterol significantly enhanced the growth performance of turbot when they fed with high plant protein diets. It is appealed that genetically modified plant ingredients are accessible in the market of certain countries ensured to have less ANFs and balanced with EAAs (Daniel *et al.*, 2016b) [90] which induce the growth (Glencross *et al.*, 2003) [91] and protein retention (Brown *et al.*, 2003) [92]

in fish.

Apart from the partial replacement of fish meal using the plant proteins, following authors also suggested that fish can be fed solely with plant proteins without affecting its growth and feed intake. Goda *et al.* (2007) [21] reported that when Nile tilapia (*Oreochromis niloticus*) and tilapia galilae (*Sarotherodon galilaeus*) received soybean meal and extruded full-fat soybean were able to completely replace dietary fish meal when supplemented with DL-methionine and L-lysine. El-Saidy *et al.* (2003) [93] reported that plant protein mixture containing 25% soybean meal, 25% cottonseed meal, 25% sunflower meal and 25% linseed meal, and 0.5% of both methionine and lysine were able to replace the fish meal completely in the diet of for Nile tilapia. Lee *et al.* (2010) [94] reported that plant based diets consisting of corn gluten, yellow soy protein concentrate and wheat gluten meal supplied with limiting amino acids and highly available inorganic phosphate when fed to rainbow trout replaced 100 % of fish meal without affecting the growth performance and feed utilization. The combined results from the previous workers showed that one can prepare feed solely with the plant based protein sources without addition of fish meal when the aforesaid advocated situations are met. Table 2 also showed the lists of some studies supporting that complete fish meal replaced by plant protein in fish diets did not affect the animal's performances.

Table 2. A List of some studies supporting that complete fish meal replaced by plant protein in fish diets did not affect the animal's performances

S. No	Species studied	Plant Ingredients used	Supported inclusion level	Remarks	References
1	Gilthead sea bream	Mixture of corn gluten meal, wheat gluten, extruded peas, rapeseed meal) balanced with EAAs	100%	Improved the protein deposition than those of fish meal based diet.	Gomez-Requeni <i>et al.</i> (2004) [38]
2	Nile tilapia	Mixture of plant protein sources	100%	No adverse effect on growth performances. Around 36% of the feed production cost was reduced.	Liti <i>et al.</i> (2006) [22]
3	Abalone	Soybean combined with either corn gluten meal or silkworm pupae meal	100%	Growth performances were not interfered.	Cho (2010) [95]
4	Rainbow trout	Mix of corn gluten, yellow soy protein concentrate and wheat gluten meal supplied with limiting EAAs and inorganic phosphate	100%	No apparent reduction in growth performance and feed utilization	Lee <i>et al.</i> (2010) [94]
5	Rainbow trout	Protein from plant protein concentrates with multiple EAA supplementations and using krill meal and the water soluble fraction of krill as feed attractant.	100%	No adverse effect on feed intake or growth.	Zhang <i>et al.</i> (2012) [65]
6	Siberian sturgeon	Mix of soybean meal and wheat gluten meal with crystalline EAAs and mono-calcium phosphate	100%	No adverse effects on growth and protein utilization.	Yun <i>et al.</i> (2014) [96]

Effect of plant proteins on protein retention in fish

Previous studies display that fish fed with plant proteins reduces the protein retention in fish. The likelihood reason could be lack of one or more EAAs in the plant proteins as reported in black tiger shrimp (Richard *et al.*, 2011) [39], tilapia (Fontainhas-Fernandes *et al.*, 1999) [69]; Atlantic salmon (Berge *et al.*, 1998; Sveier *et al.*, 2001) [36-37], rainbow trout (Gomes *et al.*, 1995; Adelizi *et al.*, 1998; De Francesco *et al.*, 2004) [30, 9, 37] or due to the results of poor metabolic adaptation of liver to higher plant proteins (Panserat *et al.*, 2009) [97]. Berge *et al.* (1999) [98] reported that plant proteins without supplementation of methionine reduced the feed conversion in Atlantic halibut. Panserat *et al.* (2008) [99] have proven that fish meal substituted with plant proteins decreases

the protein biosynthesis in in rainbow trout. Hansen *et al.* (2007) [50] showed that excess plant proteins reduce the protein retention in Atlantic cod. Lie *et al.* (2011) [100] reported that when Atlantic cod fed with higher levels (75%) of plant proteins affected the anabolic pathways of protein. It was also reported that turbot fed diet containing the highest level of plant proteins resulted in the higher rate of protein catabolism and resulted in the lower levels of N retention (Fournier *et al.*, 2004) [35].

Though some studies showed that plant proteins in the diet reduce protein retention of fish, following studies on the other hand suggested that plant protein may improve the protein retention in fish. The plausible explanation for the progressed protein retention may be due to the supplementation of EAAs

to the plant diets (Berge *et al.*, 1999)^[98]. Rolland *et al.* (2015)^[101] reported that pea protein concentrate supplemented with crystalline amino acids which include lysine, methionine and threonine that mimic just like that of fish meal enhanced the protein synthesis in rainbow trout. The rate of protein retention increases with higher rate of N retention and lesser rate of ammonia excretion in fish (Gomes *et al.*, 1993; Cheng *et al.*, 2003)^[102, 47]. Obirikorang *et al.* (2015)^[103] stated that tilapia fed with palm kernel meal reduced the ammonia excretion rates. Gomez-Requeni *et al.* (2004)^[38] demonstrated that gilthead sea bream received the mixture of corn gluten meal, wheat gluten, extruded peas and rapeseed meal balanced with indispensable amino acids improved the protein deposition than that of fish meal based diet. The activation of target of rapamycin (TOR) signalling is essential for the protein synthesis (Laplante and Sabatini, 2012)^[104]. Maggot meal is a high-quality plant protein source; it found that fish diets supplemented with maggot meal activated the TOR signal pathway resulting in the improved protein synthesis in turbot (Wang *et al.*, 2015)^[105]. It is also possible to agree that negative effect of plant proteins on protein retention in fish can be minimised by providing with certain dietary conditions which are discussed as follows: Espe *et al.* (2007)^[9] agreed that plant proteins fed with 5% of fish meal, 5% fish soluble and 3% squid hydrolysate had same responses for protein and lipid retention equal to that of fish meal based diet in Atlantic salmon. Sarker *et al.* (2012a)^[106] showed that supplementing citric acid and fatty acid in the plant based diet drastically improved the N retention in fish, thereby decreasing the N excretion. Dietary supplementation of phytase improves the protein utilization and nutrient deposition in Nile tilapia (Liebert and Portz, 2005)^[107]. Zheng *et al.* (2014)^[108] reported that lower molecular weight fish protein hydrolysate improved the protein retention in Japanese flounder when fed high plant protein diets.

Effect of plant proteins on nutrient digestibility and utilisation in fish

Previous reports have appealed that plant proteins fed to fish affect the nutrient digestibility (Fontainhas-Fernandes *et al.*, 1999; Chong *et al.*, 2002; Gaylord *et al.*, 2004; Santigosa *et al.*, 2008; Richard *et al.*, 2011; Santigosa *et al.*, 2011a; Santigosa *et al.*, 2011b; Li *et al.*, 2013)^[69, 109, 110, 111, 39, 112, 113, 114]. This can be explained that as plant proteins contain ANFs which hinder the digestibility of nutrients or excess levels of fiber or changes in the intestinal micro flora with regard to feeding plant proteins. Gaylord *et al.* (2004)^[110] suggested that utilisation of amino acid for the plant ingredients varies; they are less than fish meal. Richard *et al.* (2011)^[39] showed that black tiger shrimp fed with plant proteins (mixture of corn gluten meal, rapeseed meal, sorghum and wheat gluten) replacing 100% fish meal lowered the leucine digestibility of as much as 26%. Li *et al.* (2013)^[114] reported that digestibility and bio-availability of plant proteins was lesser in the channel catfish when fed with plant ingredients which include corn gluten meal, distillers dried grains with soluble, and canola meal. Fontainhas-Fernandes *et al.* (1999)^[69] tested that tilapia fed on sole plant protein sources had lower digestion than those of fish meal based diets. In the study by Chong *et al.* (2002)^[109], the anti-protease inhibitors for protein digestion was identified in Discus when fed with higher levels of soybean meal, wheat meal and winged bean. It was reported that activity of digestive and absorptive enzymes were lower in grass carp when fed with high-level of plant proteins; but it

got reversed when supplemented with lysine and methionine (Jiang *et al.*, 2016)^[115]. Santigosa *et al.* (2008)^[111] noticed that when Sea bream was fed with plant protein sources reduced its digestive activity; but growth rates were similar to that of fish meal diets as compensation mechanisms were discovered in this fish i.e. increase in the relative intestinal length (RIL) and up-regulation of trypsin activity. Santigosa *et al.* (2011a)^[112] and Santigosa *et al.* (2011b)^[113] reported that Sea bream and Rainbow trout fed with plant protein sources delayed the intestinal nutrient absorption.

Although some earlier reports show the negative influences of nutrient digestion in fish with response to dietary plant proteins, the evidences are also available that plant based protein feeding had no adverse effect on the digestibility of nutrients in fish. Hansen *et al.* (2006)^[116] reported that Atlantic cod may be fed with plant based feeds up to 44 % without any adverse impact to nutrient digestibility. Bonaldo *et al.* (2011)^[8] showed that turbot fed with higher plant protein (mixture of soybean meal, wheat gluten meal and corn gluten meal) in the diet did not cause the digestibility of ingredients and gut histology. Da *et al.* (2013b)^[117] found that groundnut cake can be used to replace fish meal with no effect on the diet digestibility in striped catfish fingerlings. Sampaio-Oliveira and Cyrino, 2008^[118] confirmed that 100 % plant proteins offered best protein digestibility when fed together with attractants than that of 50 % of plant proteins: 50 % animal proteins to the carnivorous fish, largemouth bass. Soybean meal (SBM) is a promising protein source for fish meal replacement (Lemos *et al.*, 2000)^[119]. But it can't be used in higher quantities as they contain anti-nutritional factors (Rumsey *et al.*, 1994; Anderson and Wolf 1995)^[120-121] as well as imbalanced amino acid profile (Wilson, 1989; Floreto *et al.*, 2000)^[122-123]. However it is claimed that solid-state fermentation strategy using micro-organisms improves the metabolites along with enzymes and antibiotics that improve the digestion and metabolism in animals (Holker and Lenz, 2005)^[124]. It is worth noting that there is variation in digestibility of every ingredient. The selection of plant ingredients is therefore important due to this variation in the digestibility of different plant sources. Da *et al.* (2013a)^[125] reported that apparent protein digestibility of different plant ingredients such as broken rice, maize meal; soybean meal, cassava leaf meal and sweet potato leaf meal were not same in the striped catfish. Papatryphon *et al.* (2001b)^[126] reported that supplementation of phytase in the feed increased the apparent protein digestibility in the striped bass. Santigosa *et al.* (2011a)^[112] reported that 75% of the proteins and 66% of lipids sources can be replaced by the vegetable sources in gilthead sea bream without compromising the digestive processes. Dietary organic acid reduces the pH in the stomach which results in the enhancing of pepsin activity and protein digestion in animals (Mroz *et al.*, 2000)^[127]. Zhang *et al.* (2016)^[74] showed that large yellow croaker fed with high plant protein diets together with citric acid had positive influence on the digestive functions as well as lowering the intestinal oxidation. Dietary xylanase is claimed to increase the microbiota and nutrient digestion in the animals (Dumitrescu *et al.*, 2011)^[128]. Jiang *et al.* (2014)^[23] reported that xylanase supplementation in plant protein-enriched diets increases the growth performance, intestinal enzyme activities as well as intestinal microflora in Jian Carp.

It can also be noted that the digestion, utilisation and bio-availability of nutrients also depend on the forms and nature of dietary components. The usage of dietary crystalline

methionine was very common in the fish feed to improve the amino acid bio-availability in fish. However, it is claimed that they have more leaching which results in the lower bio-availability to the animals (Yuan *et al.*, 2011)^[129] than that of intact protein (Peres and Oliva-Teles, 2005; Hauler *et al.*, 2007; Dabrowski *et al.*, 2010)^[130-132]. Jost *et al.* (1980)^[133] demonstrated that oligo-methionine is water-insoluble in nature. The higher bio-availability of oligo-methionine has been observed in the rats (Chiji *et al.*, 1990; Hara and Kiriyama, 1991; Kasai *et al.*, 1996)^[134-136]. It additionally discovered that oligo-methionine notably helped in the growth performance and feed utilization of white shrimp, *Litopenaeus vannamei* as compared to crystalline methionine (CMet) when fed with plant protein-enriched diets (Gu *et al.*, 2013)^[137].

Effect of plant proteins on bio-availability and utilization of micronutrients in fish

Previous reports of fish showed that fish fed with plant protein have increased the loss of certain vitamins (riboflavin, niacin, pantothenic acid and vitamin B12) content of fish (Bell and Waagbo, 2008)^[138]. Vitamin B is very essential for the proper metabolism of animals. Hansen *et al.* (2015)^[139] advocated that supplementation of plant based diets need to be supplemented with the several B vitamins as their availability is low in plant proteins. Cheng *et al.* (2016)^[140] demonstrated that plant proteins lower certain mineral (phosphorus) content in yellow catfish due to very low bio-availability of P in the plant proteins. Welker *et al.* (2016)^[143] highlighted that ANFs present in the plant proteins often make micronutrients including zinc unavailable to the fish which results in zinc deficiency. Diets prepared with plant ingredients are often resulting in low P bioavailability to the animals (Goda *et al.*, 2007)^[121]. Approximately 75 % of the P from plant feedstuffs exists as the phytate-phosphorus that has low digestibility in fish due to lack of enzyme phytase (Lall, 1991)^[141]. Therefore, animals grab less P from the plant ingredients and create P pollution in the environment in addition to eutrophication (GESAMP, 1996)^[142]. Kaushik *et al.* (2004)^[48] noticed that European sea bass fed with plant protein increased the loss of N and P in it. The same tendency was observed by Lund *et al.* (2011)^[11] in trout, he found that higher plant proteins lowered the total P content in animals.

Some authors also suggested that supplementing plant protein showed no poor effect on the bio-availability of micronutrients. A number of authors explained these as a result of adopting certain dietary manipulations which are discussed as follows: Lee *et al.* (2010)^[94] suggested that fish bone meal can be used as a source of calcium and phosphorus source to fish when fed on plant proteins without any detrimental effect on the bio-availability of certain minerals. It was also found that addition of meat and bone meal (MBM) at the rate of 7% to plant-protein-based diets improved the P utilization in the Nile tilapia (Suloma *et al.*, 2013)^[143]. It is advised that fermentation of plant ingredients may improve micro-nutrients (Vitamin A and B) and essential amino acids content (Weng and Chen, 2010)^[144]. Therefore, one can expect that fermented plant ingredients can lower the amounts of micro-nutrients and EAA required in the feed that includes higher plant proteins. Cheng *et al.* (2003)^[47] reported that plant proteins supplemented with lysine minimised the dietary protein level in rainbow trout diets, and reduce ammonia nitrogen and soluble P excretion. Cheng *et al.* (2016)^[140] also recommended that improving the P utilization decreases the P and N pollution to the pond environment. Supplementation of

phytase in the feed increased the P absorption in the striped bass and *Morone saxatilis* (Papatryphon *et al.*, 2001b)^[126]. It is reported that increase in the calcium/phosphorus in the diet had reduced the phytase activity in swine, poultry (Lei *et al.*, 1994; Qian *et al.*, 1996; Li *et al.*, 1999)^[145-147] as well as in fish (Vandenberg *et al.*, 2012)^[148]. The reason may be because of the formation of insoluble calcium-phytate complexes which make dietary supplemented phytate insensitive to phytase (Qian *et al.*, 1996)^[146]. Liebert *et al.* (2005)^[107] reported that Nile tilapia that received microbial phytase notably increased the P utilization in the plant based low P diets. Organic acids have a positive influence on the animal performances; it increases the P absorption in the small intestine (Ravindran and Kornegay, 1993)^[149]. Øverland *et al.* (2000)^[25] suggested that organic acids lower the pH which increases the ability of P to bind with various cations and act as a chelating agent, which will further increase the solubility of P and phytate and absorption in the small intestine. Sarker *et al.* (2012a)^[106] encouraged that supplementing with citric acid (CA) and fatty acid (FA) in the plant based diet significantly increased P retention in yellowtail, thereby decreasing the P excretion. Besides, it also reduced the dependence of supplementary inorganic phosphates (polluting nutrient) to the diet. An addition of citric acid and formic acid to the plant protein source-based diets enhances the bioavailability and retention of certain minerals (Ca, Mg, Na, K, Zn and Mn) in fish (Sugiura *et al.*, 2000; Sarker *et al.*, 2012b)^[150-151]. Zhang *et al.* (2016)^[74] reported that large yellow croaker fed with high plant protein diets together with citric acid had positive influence on the mineral availability.

The utilization and absorption of minerals also depend on chemical form of minerals i.e. chelated trace minerals or inorganic trace minerals. Previous reports suggest that chelated trace minerals have more bio-availability to animals than to inorganic trace minerals (Shao *et al.*, 2010; Lin *et al.*, 2013; Katya *et al.*, 2016)^[152-154]. The higher availability of trace minerals from chelated sources is favourable because of their high stability in the digestive tract, less susceptibility and less interaction to bind with other organic molecules (Bharadwaj *et al.*, 2014)^[155]. Prabhu *et al.* (2014)^[156] clearly demonstrated that plasma mineral levels got improved after postprandial stage in rainbow trout fed with complete plant ingredients based diet supplemented with di-calcium phosphate.

Effect of plant proteins on biochemical compositions in fish

Biochemical compositions of aquatic animals change according to the diet and its nutritional composition (Zhou and Yue, 2010)^[157]. Earlier reports suggested that plant proteins in the diet affect the biochemical compositions in fish. Lund *et al.* (2011)^[11] have established that trout that received excess plant protein in the diet induced higher excretion of ammonium-nitrogen, indicating the imbalance of dietary essential amino acid composition in plant proteins. It was found that excess supplementation of plant proteins in the diet resulted in the decreased liver size, plasma triacylglycerol concentration (TAG) and lipid productive value (LPV) in Atlantic cod (Espe *et al.*, 2010; Hansen *et al.*, 2011)^[158, 10]. Tocher *et al.* (2003)^[159] also showed that Atlantic salmon fed diets excessive in plant ingredients increased the liver TAG concentrations. Reports are also claiming that plant protein sources in the diet result in the reduction in growth and have

hypocholesterolemic effect. This may be due to use of high amounts of plant ingredients which contain negligible amounts of cholesterol (Yun *et al.*, 2011) ^[160]. It was also reported that plant protein in the diet accelerated the fat deposition in European sea bass (Kaushik *et al.*, 2004) ^[48]. There are some available reports showing the negative performances in the biochemical compositions in response to plant protein intake, some studies are also claiming that fish can be fed with plant protein without affecting the biochemical compositions. Rodiles *et al.* (2015) ^[58] recorded that 30% fish meal replacement using the plant protein sources such as soybean meal, soybean protein concentrate and wheat gluten meal no longer offered any changes in the proximate composition of muscle, fatty acid profile and plasma, hepatic and muscular metabolites parameters in the Senegalese sole. Hansen *et al.* (2007) ^[50] noticed no impact in the whole body, liver, muscle proximate compositions, blood parameters as well as health status in Atlantic cod when fed with high plant proteins. Jiang *et al.* (2013) ^[161] showed that cottonseed meal that replaced 64 % of fish meal had not compromised the body composition of crab. An interaction of dietary lysine and methionine in the protein and lipid metabolism of fish has been recorded in the many fish species (Walton *et al.*, 1984; Marcouli *et al.*, 2006; Espe *et al.*, 2008) ^[162-164]. Lysine and methionine are required for the biosynthesis of carnitine and energy metabolism in fish (Tanphaichitr *et al.*, 1971) ^[165]. Report says that supplementation of plant sources in the diet often lack these two essential amino acids for growth. It also observed that plant based feeds without these two EAA often results in the inability of fish to obtain these two EAA for the maximal growth for the tissue protein accretion (NRC, 1993) ^[166]. Hansen *et al.* (2011) ^[10] also endorsed that lysine supplementation in plant sources influences the lipid metabolism by lowering the lipid deposition in Atlantic cod. Gaylord *et al.* (2007) ^[167] reported that dietary methionine to plant based diets reduces intraperitoneal fat composition in rainbow trout. Liland *et al.* (2015) ^[168] reported that Atlantic salmon fed diets high in plant ingredients with processed poultry and porcine by-products reduced the liver triacylglycerol (TAG). Docosahexaenoic acid (DHA) is an essential fatty acid and have role in the growth, metabolism and health of the animals (Bureau *et al.*, 2008) ^[169]. Yu *et al.* (2015) ^[42] showed that compositions of DHA were altered in sea cucumber when fish meal was replaced with plant proteins. Dietary cholesterol is important for shrimp for the growth and survival of crustaceans (Sheen *et al.*, 1994; Smith *et al.*, 2001) ^[170, 171]. Previous results in the literature suggesting that phytosterol from plant proteins may be used as a cholesterol substitute for shrimp (Gong *et al.*, 2000; Roy *et al.*, 2006; Morris *et al.*, 2011) ^[172-174], which recommend that plant proteins in the diet are able to reduce the cholesterol supplementation in the shrimp diet.

Effect of plant proteins on flesh quality in fish

In the literature there are many reports for the evaluation of plant proteins as potential feed ingredients in the diet for numerous fishes; but only few reports are on its organoleptic attribute to fish. Previous authors reported that dietary plant proteins lower the flesh quality of fish (Alami-Durante *et al.*, 2010; Valente *et al.*, 2016) ^[44, 13]. De Francesco *et al.* (2004) ^[6] also reported that fillets and some organoleptic properties of flesh are affected in rainbow trout when fed with plant proteins for long term duration. Even though some earlier

reports show the adverse effect of dietary plant proteins on flesh quality in fish, a number of reports are also available in the literature suggesting that feeding plant proteins have not affected the flesh quality in fish. It was demonstrated that substituting fish meal with high level of plant proteins had no detrimental effects on the texture properties and sensory attributes (flesh quality) in gilthead sea bream (Matos *et al.*, 2012; Matos *et al.*, 2014) ^[175-176]. The similar results were also obtained in Atlantic salmon when fed to plant proteins (Johnsen *et al.*, 2011) ^[177]. Cabral *et al.* (2013) ^[56] showed that fish meal replaced via plant protein sources up to 75% has not affected the flesh quality of Senegalese sole. Hisano *et al.* (2016) ^[178] reported that plant proteins (corn gluten meal) fed to pacu did not affect fillet quality in pacu. Kaushik *et al.* (1995) ^[179] and Aoki *et al.* (1996) ^[180] exhibited that plant protein-based diets fed to fish did not alter the organoleptic or flesh quality of fish. L-Carnitine is the AA considered to have role in the growth promotion of animals, concurrently reduces the fat accumulation in the fish tissues by increasing the lipid oxidation for the utilization of the energy from the lipids (Harpaz, 2005; Ozório, 2009) ^[181-182]. It was reported that high levels of plant proteins with L-Carnitine supplementation gave growth-promoting effect as well as decreased the intraperitoneal fat ratio and whole body lipid contents of the silver perch (Yang *et al.*, 2012) ^[183]. Excess fat deposition of fish leads to poor flesh quality and less consumer preference. Therefore, it might be useful to supplement the L-carnitine to make sure that greater proportion of the energy is taken by dietary lipids, which results in less fat deposition. Also, L-carnitine is usually synthesized from lysine and methionine, which can be deficient in plant proteins (Yang *et al.*, 2012) ^[183]. Therefore, supplementing L-Carnitine is supportable while increasing the plant proteins in the fish diets; probably this could spare the lysine and methionine which are the most important for the animals.

Effect of plant proteins on immune and stress parameters in fish

Previous authors recommended that increasing plant proteins in the diet of some carnivorous fish may disturb the immunity as they contain ANFs (Hardy, 2010) ^[2]. Vilhelmsson *et al.* (2004) ^[184] reported that rainbow trout fed on high plant proteins resulted in the over-expression of hepatic genes involved in stress and welfare in rainbow trout. Ferrara *et al.* (2015) ^[185] reported that soybean meal substituted with 40 % of fish meal induced inflammatory reaction in the gut of sharp snout sea bream. Baevefjord and Krogdahl (1996) ^[186] showed that Atlantic salmon fed with soybean meal induced the enteritis in distal intestine. Overturf *et al.* (2012) ^[187] demonstrated that rainbow trout fed with plant-based diet down regulated the cell survival and turnover. Sissener *et al.* (2013) ^[188] reported that simultaneous replacement of fish meal and fish oil elevated the stress in Atlantic salmon. In contradiction with the earlier findings aforementioned, some studies also suggested that fish meal can be replaced with plant proteins without affecting the immune performances of aquatic animals. Hansen *et al.* (2006) ^[116] recommended that Atlantic cod may be fed with plant based diets of up to 44 % without any adverse impact on intestinal or liver functions. Sitjà-Bobadilla *et al.* (2005) ^[189] also mentioned that immune and anti-oxidant status of gilthead sea bream fed with 50% of plant proteins were not immunosuppressed. Some studies have also validated that excess plant protein in the diets did not affect the immune and

stress responses in fish when some dietary tactics were followed. Probiotics are used in the aquaculture to elicit the immune responses in fish. Merrifield *et al.* (2010)^[15] reported that rainbow trout fed high plant proteins intercropped with probiotics supplementation undoubtedly prompted the immune and strain responses. Some authors are also suggesting that negative effect of animal with dietary plant proteins can be alleviated by modifying the gut microbiota (Wiggins, 1984; Cummings *et al.*, 1986)^[190, 191]. Taurine is an AA proved to have various roles together with immunoregulation and detoxification (Motawi *et al.*, 2007; Gulyasar *et al.*, 2010)^[192-193]. Li *et al.* (2016)^[19] mentioned that dietary plant protein diets together with taurine improved the immunity as well as decreased the ammonia levels in yellow catfish. Panserat *et al.* (2009)^[97] reported that rainbow trout fed with higher plant protein diets did not induce the stress parameters. Dietary nucleotides are claiming to influence the immunity and stress in fish (Nageswari and Daniel, 2015)^[194]. Guo *et al.* (2016)^[18] reported that nucleotides fed along with low fish meal diets improved the immune responses and disease resistances against challenge with pathogenic bacteria (*Vibrio parahaemolyticus*) in pacific white shrimp. Haematological parameters are reliable indicators to assess the health status of fish. Hisano *et al.* (2016)^[178] manifested that plant proteins (corn gluten meal) fed to pacu did not affect the haematological parameters in pacu. Soltanzadeh *et al.* (2016)^[195] reported that faba bean replaced with fish meal 10 % did not show negative effect on survival, haematological, and serum biochemical parameters in beluga. Kpundeh *et al.* (2015)^[17] reported that GIFT tilapia fed with plant proteins along with fish meal did not affect the haemato-immunological parameters. Microalgae contain rich amount of essential molecules of poly unsaturated fatty acids (PUFA), natural antioxidant molecules as well as carotenoids (Sporale *et al.*, 2006; Sousa *et al.*, 2008)^[196-197]. Sheikhzadeh *et al.* (2012)^[16] showed that rainbow trout fed with microalgae in the diet improved the immune and stress parameters. But the production of microalgae in huge amounts and its storage is difficult at the farms. Daniel *et al.* (2016c)^[198] advocated that using the photo bioreactor, microalgae would be produced in large scale with greater yield, which can be further dried using the freeze drier and it can be potentially used to partially replace the costlier feed ingredients, including fish meal in the diets for aquatic animals.

Recommendations for further studies

In future, high or complete supplementation levels of plant proteins are likely to be held in the fish feed. In that connection, future research is required in the following areas: Diets prepared with high concentrations of plant ingredients requiring processing methods to alleviate the ANFs present in them. Though many processing techniques claim to remove the ANFs present in the plant feedstuffs, it varies with ingredients. Therefore, the best processing methods should be standardised for all the plant ingredients. Future studies should also address the issues associated with increased utilization of plant proteins by fish; its negative effects at high concentration levels should be recorded and proper technology should be standardised to alleviate these effects. It is possible that fish that reared in consuming fish meal free diets may not be adequately store the EAA content in the tissues. Therefore, the feed should be designed in such a way that animals can reserve the EAA profiles in the tissues that

are equal to what they can hold when fed with fish meal based diets. There is no doubt that plant ingredients can reduce the feed costs; but still feed millers would be motivated to prepare feeds at lower costs. Therefore, plant ingredients should be ranked according to the basis of their costs and much priority of the research should be focussed on cheaper cost ingredients. The molecular tools should intervene in the feed nutrition study when replacing fish meal in the fish diets. In response to dietary intake of plant-based diets, researchers should study the up regulation and down regulation of genes connected to the digestion, metabolism and growth processes. Microarray studies (whole genome analysis) could be done in fish tissues when they are fed plant diets. These kinds of studies would certainly help in identifying the effect of plant diets on cellular processes in fish for the dietary standardisation limits which don't disturb the cellular processes in fish. Earlier authors studied the genotypes versus diet interactions in the European sea bass with regard to feeding plant based diet (Le Boucher *et al.*, 2011)^[199]. Through these types of approaches it is possible to identify the fish species that will provide higher positive response to the plant based diets. For those species, fish feed can be doubtlessly prepared with higher portion of plant based ingredients to prepare low cost commercial feeds. Previous findings have reported that the alternative feeds fed with plant protein ingredients (irrespective of their low N and P contents) instead of fish meal increased the plant and fish yield which ensured more profitability in aquaponics based intensive fish rearing systems (Medina *et al.*, 2016)^[200]. These sorts of results are really encouraging, but similar research should also be focussed over other intensive fish rearing practices.

Concluding remarks

Based on the available reports in the literature, the effect of dietary plant based ingredients on fish has been very well discussed in this review. It seemed that several authors working in the fish feed research have agreed on the inevitable requirement of placing the plant based protein ingredients to replace fish meal in the diet for commercially cultivable aquatic animals. Taking this board, we may hope that fish meal will no longer be a part of the fish diets in future. Although there is a major challenge in the expansion of plant ingredients, it is justified by many authors that through proper dietary tactics fish can be fed with excess plant protein without any negative performances. Fish meal may also be balanced with some other micronutrients (vitamins and minerals) or biologically active compounds other than specified in the paper (Barrows *et al.*, 2008; Barrows *et al.*, 2010)^[201-202], which will be soon a further topic of debate in this area and it is likely to be claimed that it may be the reason for the superior nature of fish meal than that of plant meals. But it can be expected that through the development and standardisation of appropriate dietary strategies, the expansion of plant based sources in the fish diets can be supported. In overall, author concludes that the information given in this paper would support the feed formulators for the development of cost effective diets without fish meals with maximum addition of plant feedstuffs in the aqua feeds.

Conflict of interest statement

Author declares that there is no conflict of interest in the manuscript.

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