Chapter

Legumes, Sustainable Alternative Protein Sources for Aquafeeds

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Abstract

Aquaculture produce a great portion of aquatic derived proteins for human in the world. It has the highest and the fastest growth rate among the protein producing industries. Fish meal (FM) is the main and the most expensive ingredient for aquafeeds production. It provides protein, essential amino acids, energy, minerals and vitamins in aquafeeds. Given the current rapid development of aquaculture industry the competition for limited global supplies of FM may reduce its availability and elevate its price. Thus, finding high quality, economic and environmentally friendly alternative protein sources (APS) for aquafeeds production is vital for sustainability of the aquaculture industry. Among various APS, legumes have been proved to be promising APS because they have medium protein content with suitable amino acid profile, high digestible protein and energy levels, and appropriate minerals and vitamins for the most cultured aquatic species. They also are cost-effective and highly accessible. However, they contain various anti-nutritional factors that may reduce feed palatability and may negatively affect growth and health of cultured aquatic animal species. This chapter provide information regarding legumes and their derivatives as APS, their nutritional quality and their potential drawbacks. In addition, strategies for increasing the efficiency of legumes in aquafeeds are reviewed and discussed.

Keywords: additives, anti-nutritional factors, aquaculture, nutrients digestibility, essential amino acids

1. Introduction

The aquafeed market is estimated to account for USD 50.6 billion in 2020 and with compound annual growth rate of 7.2%, it is projected to reach USD 71.6 billion by 2025 [1]. Two main factors amplify such a lucrative revenue in aquafeed market including increase in global seafood consumption (122% from 1990 to 2018) and fast grow rate in aquaculture production (527% from 1990 to 2018) [2]. In fact, the annual growth rate of aquaculture industry was about 10% during the 1990s and about 5.8% annually from 2000 to 2018 indicating aquaculture is the fastest growing food production sector in the world [3]. According to Food and Agriculture Organization of the United Nations [2] the aquaculture accounted for 52% (54.3 million tonnes (MT)) of global fish production that means this industry supplied 17% of total animal proteins for the global population [2]. About 70% of aquaculture production relied on the aquafeeds, which is the main expenditure (∼ 40%–70% of the total expenses) and the largest input in this industry [4, 5]. Thus, the development of the aquafeed industry along with the improvement of feed efficiency are prerequisite to achieve the projected aquaculture production. On the other hand, aquafeed production industries mainly depend on marine-derived ingredients namely fish meal (FM) and fish oil (FO), but increasing demands for these marine-derived feedstuffs along with overexploitation and/or static tendency in capture fisheries of small pelagic fish resulted in uncertain supply and the inflation of their prices that adversely affect the profitability margins of aquaculture [6, 7]. It has been reported that about 70% to 80% of all produced FM is used in aquafeed industry [3]. Thus, seeking out environmentally friendly and economic alternative ingredients for substitution of FM and FO in aquafeeds formulation is a fundamental goal for aquaculture sustainability [7].

An alternative feedstuff for FM should possess some properties such as high availability, commercial competitive cost as well as ease of shipping and storage [8]. In addition, by considering nutritional aspects, an alternative protein source (APS) should have low quantities of fiber ($< 6\%$), carbohydrates including starch ($< 20\%$) and non-starch polysaccharides (NSP)($< 8\%$), anti-nutritional factors (ANF), high digestible protein level ($\geq 48\%$), appropriate essential amino acid (EAA) profile (arginine > 3, lysine > 3.5, methionine > 1.5, threonine $> 2.2\%$ of total AA profile) and suitable palatability [6, 8]. On the other hand, it has been predicted that the application of FM in aquafeeds will be dropped to under 10% in some most popular aquaculture species such as omnivorous (*e.g.* carp, catfish and tilapia, 1-2% FM) and carnivorous fish species (*e.g.* marine fish, salmon and trout, 5-10% FM) [9]. Such tremendous shift from FM to APS in aquafeeds has resulted in new challenges in performance, feed utilization, welfare, and final product quality of cultured aquatic animal species [6].

Among alternative plant protein sources (APPS), legumes are the most abundant protein rich ingredients for applying in aquafeeds [10]. However, these APPS have several drawbacks such as a wide range of protein contents, EAA imbalances or inadequacies (*e.g.* sulfur amino acids including cysteine, methionine and taurine), low bioavailability of some minerals or microelements as they bounded with phytic acid (*e.g.* phosphorous, iodine, calcium and selenium) and the presence of high amounts of various ANF (*e.g.* antitrypsin factors, phytates, saponins, and polyphenols), which consequently impose some challenges in their use in aquafeeds formulations [8, 11]. Although some processed protein derivatives of legumes such as soy protein concentrate, soy protein isolate or pea protein isolate contain high protein levels (65-90%) and have low ANF concentrations, but they are too expensive to be used in most aquafeeds. In addition, the agriculture industry also restricted to develop production of these APPS without putting extra stress on land, water, and phosphorous resources. In this chapter it was aimed to highlight the opportunities and challenges in application of legumes as APPS and providing some strategies for enhancing their efficiency in aquafeeds.

2. Legumes, alternative protein sources for aquafeeds

The family Fabaceae (Leguminosae) or commonly named legumes are the third largest family of flowering plants with almost 770 genera and over 19,500 species [12, 13]. The largest and the most important economically subfamily of the legumes, are the Faboideae, which are the source of primary crops including dry seeds (*e.g.* lentils, broad beans, beans and peas), flavoring plants (*e.g.* carob and lupins), fodder plants (*e.g.* alfalfa) and oilseeds (*e.g.* peanut or groundnut and soybeans). The seeds are the most important part of the legumes that used as ingredients for aquafeeds manufacturing. In this section, it has been tried to introduce the most important legumes used in the aquaculture industry. In 2020, the global production of some legumes such as peanut, dry pea, chick pea, cow pea, lentil and lupins were 48.757, 48.75, 14.184, 14.246, 8.786, 5.734 and 1.01 MT, respectively [14].

2.1 Soybean (*Glycine max*)

Soybean (*Glycine max*) is the leading oilseed crop in the world and occupies the first place as a global APS for FM due to its large amounts of protein (~ 40%) and reasonable EAA profile as well as its most availability in the global market [8]. Moreover, owing to genetic engineering technologies and plant biotechnology available for the crop, the output traits of soybean also improved for aquaculture purposes [15]. The global production of soybean was estimated to be 336.7 MT in 2020 and projected to reach to 371.3 MT in 2030 with annual growth rate of 1.8% from 2020 to 2030. A huge amount of this crop used for oil extraction that yield a cake with high levels of protein that will be processed to a wide range of soy products including soy flour, soybean meal (SBM), full-fat SBM, soy protein concentrate (SPC), soy protein isolate (SPI), soy protein hydrolysate (SPH) and fermented SBM (FSBM).

2.2 Soybean meal

Unprocessed SBM contains 30-50% protein, about 30% carbohydrates including oligosaccharides (*e.g.* stachyose and raffinose), starch and NSP (*e.g.* cellulose, hemicellulose, and pectins; **Table 1**) [16–18]. Except for cystine, the amounts of the EAA (mainly lysine, methionine and threonine; **Table 2**), taurine and tyrosine in SBM are generally lower than FM [8]. The protein level and EAA content can be enhanced by chemical processing of soy flakes to SPC and SPI or through hydrolysation and fermentation of SBM. In addition, high levels of ANF in SBM can be reduced by conventional processing (*e.g.* heat, autoclaving, and use of solvents).

2.3 Soy protein concentrate

Soy protein concentrate is commonly obtained by fractionation of SBM through aqueous alcohol that not only enhance protein content (~ 70%) [19–21] by extracting carbohydrates but also remove ANF (*e.g.* saponin), soluble oligosaccharides and fiber [21–23] that eventually increase its palatability and protein digestibility [24]. Soy protein concentrate has been getting more attention in aquafeed industry because of its well-balanced AA profile compared to other APPS [23, 25].

2.4 Soy protein isolate

Soy protein isolate is produced by refinement of SBM through a series of aqueous extractions with different pH levels that increase its protein content over 78% [26]. As, the processing of SPI does not have the aqueous alcohol extraction step, the saponin content of SPI (~0.8%) is higher than that in SPC (~0%) [27, 28]. Because of high protein content in SPI, the inclusion levels of this ingredient in aquafeeds could be lower than SBM and SPC that eventually reduce the total ANF input in aquafeed. On the other hand, it has been confirmed that the nutrients digestibility of SPI was higher than that of SPC, when it was replaced by 40% of FM in feed for rainbow trout (*Oncorhynchus mykiss*) [29].

Protein sources	Bioactive component																		
	Oxidized and polymerized lipids	Histamine and putrescine	Nitosamines	Protease/trypsin inhibitors	Amylase inhibitors	Saponins	Phytate	Glucosinolates	Sterols	Oxalate	Anti-vitamins	Polyphenols/Tannins	Lectins	Phytoestrogens	Alkaloids	Cyanogens	NSP	Oligosacharide	Antigenic proteins
Fish meal	●	●	●																
Soybean	●			●		●	●	●	●		●		●	●			●	●	●
Faba bean				●		●	●		●			●	●						●
Lupin				●		●	●		●			●		●	●	●	●	●	
Peas				●	●	●	●		●	●	●	●	●			●		●	
Pea nut				●	●		●		●			●			●				
Alfalfa						●			●		●	●		●	●				
carob seed germ meal				●		●			●			●							
Soy/Lupin/Pea/Faba/Alfalfa PC						●	●		●			●							
Soy/Lupin/Pea PI						●													
Novel varieties				●	●	●	●		●		●						●	●	●

PC: protein concentrate; PI: Protein Isolate.

Table 1.
Bioactive component present in legumes and means of alleviation.

Protein sources	Protein content(%)	Lys(CS)	Met(CS)	Cys	Arg(CS)	Trp	Leu(CS)	Ile(CS)	Phe	His	Thr(CS)	Tyr	Val	Arg/Lys (CS)	Met/Lys (CS)
	g.100 g^{-1} protein														
Fish meal	70	7.5	2.7	0.8	6.2	0.6	7.2	4.2	3.8	2.4	4.1	2.7	4.9	0.83	0.36
Faba bean	29.0	6.3 (16↓)	0.8 (70.4↓)	1.2	9.0 (45.2↑)	0.3	7.1 (1.4↓)	4.1 (2.4↓)	4.0	2.6	3.5 (14.6↓)	1.2	4.6	1.43 (72.3↑)	0.13 (64↓)
Faba bean PC	63	6.63 (11.6↓)	0.71 (73.7↓)	1.1	8.68 (40↑)	1	7.57(	4.1 (2.4↓)	4.35	2.5	3.49 (14.9↓)	3.35	4.48	1.31 (57.8↑)	0.11 (69.4↓)
Lupin (*Lupinnus angustifolius*)	33.8	4.7 (37.3↓)	0.7 (74.1↓)	1.5	11.0 (77.4↑)		6.9 (4.2↓)	4.2(=)	4.0	2.7	3.4 (17.1↓)	3.6	3.9	2.34 (182↑)	0.15 (58.3↓)
dehulled lupin (*Lupinus albus*) meal	42	5(33.3↓)	0.8 (70.4↓)	1.6	11.3 (82.3↑)		7.3 (1.4↑)	4.2(=)	3.9	2.3	3.8 (7.3↓)	4.8	4	2.26 (172.3↑)	0.16 (55.6↓)
Lupin PI	61	3.44 (54.1↓)	0.6 (77.8↓)	0.33	9.02 (45.5↑)		5.25 (27.1↓)	2.46 (41.4↓)	2.95	1.97	2.62 (36.1↓)	3.11	2.29	2.62 (215.7↑)	0.17 (52.8↓)
Fermented Lupin	40	5.57 (25.7↓)	0.82 (69.6↓)	1.4	9.75 (57.3↑)	0.83	7.3 (1.4↑)	4.6 (9.5↑)	4.25	3.3	4.1(=)	4.25	4.75	1.75 (110.8↑)	0.15 (58.3↓)
Pea seed	23.9	7.2(4↓)	1.0(63↓)	1.4	8.4 (35.5↑)		7.1 (1.4↓)	4.2(=)	4.7	2.5	3.8 (7.3↓)	3.1	4.8	1.17(41↑)	0.14 (61.1↓)
Filed pea PC	46.5	7.61 (1.5↑)	0.9 (66.7↓)	1.25	8.32 (34.2↑)	0.99	7.31 (1.5↓)	4.26 (1.4↑)	4.95	2.39	3.51 (14.4↓)	3.31	4.73	1.1 (32.5↑)	0.12 (66.7↓)
Pea PI	80	6(20↓)	0.78 (71.1↓)	0.25	7.4 (19.4↑)		7.13(1↓)	3(28.6↓)	4.75	2	3.13 (23.7↓)	3.25	3.5	1.23 (48↑)	0.78 (116.7↑)
Bambara nut		8(6.7↑)	0.64 (76.3↓)	2.41	7.48 (20.6↑)	0.6	10.2 (41.7↑)	5.45 (29.6↑)	7.69	3.86	4.43(8↑)	3.13	6.24	0.94 (13↑)	0.08 (77.8↓)
Full fat soy bean meal	36	6.3(16↓)	1.29 (52.2↓)		7.44 (20↑)	1.44	7.08 (1.7↓)	5.31 (26.4↑)	5.2	2.58	4.18(2↑)		4.97	1.18(42↑)	0.20 (44.4↓)
Soy bean (expeller)	43.5–49.3	6.3(16↓)	1.4 (48.2↓)	1.6	7.5(21↑)	1.2	7.7 (6.9↑)	4.6 (9.5↑)	5.1	2.7	3.7 (9.8↓)	3.5	4.5	1.19 (43.4↑)	0.22 (38.9↓)

Protein sources	Protein content(%)	Lys(CS)	Met(CS)	Cys	Arg(CS)	Trp	Leu(CS)	Ile(CS)	Phe	His	Thr(CS)	Tyr	Val	Arg/Lys (CS)	Met/Lys (CS)
	g.100 g^{-1} protein														
Soybean (dehulled)	53.5	6.3(16↓)	1.4 (48.2↓)	1.6	7.3 (17.7↑)	1.4	7.7 (6.9↑)	4.6 (9.5↑)	5.1	2.7	3.8 (7.3↓)	3.5	4.8	1.16 (39.8↑)	0.22 (39.8↓)
Soy PC	67–72	6.3(16↓)	1.3 (51.9↓)	1.25	7.3 (17.7↑)	1.5	7.9 (9.7↑)	4.6 (9.5↑)	5.1	2.6	4.3 (4.9↑)	3.5	4.8	1.16 (39.8↑)	0.21 (41.7↓)
Soy PI	90–92	6.0 (20↓)	1.0(63↓)		7.2 (16.1↑)	1.2	7.8 (8.3↑)	4.5 (7.1↑)	5.2	2.5	3.5 (14.6↓)		4.6	1.2 (44.6↑)	0.17 (52.8↓)
Fermented soy bean	48.9	6.2 (17.3↓)	1.6 (40.74↓)		7.8 (25.8↑)	1.23	8.2 (13.9↑)	4.91 (16.9↑)	5.5	2.66	4.1(=)		4.1	1.3 (56.6↑)	0.26 (37.8↓)
Pea nut meal (corticated-decorticated)	32–46.5	2.29 (69.5↓)	0.53 (80.4↓)		5.28 (14.8↓)	0.48	3.18 (55.8↓)	1.80 (57↓)	2.37	1.26	1.40 (65.9↓)		2.08	2.3(177↑)	0.23 (36.1↓)
Pea nut meal expeller (corticated-decorticated)	34.1–46.5	2.46 (67.2↓)	0.43 (84.1↓)		5.01 (19.2↓)	0.37	3.03 (57.9↓)	1.58 (62.4↓)	2.32	1.05	1.27 (69↓)		2.06	3.43 (313↑)	0.29 (19.4↓)
(Chloroplastic) alfalfa leaf PC	53.22	4.57 (39.1↓)	0.3 (88.9↓)		5(19.3↓)	4	8(11.1↑)	4.57 (8.8↑)	5.2	2.4	4.5 (9.8↑)		5.2	1.1 (32.5↑)	0.07 (80.6↓)
Cytoplasmic alfalfa leaf PC	69.24	5.3 (29.3↓)	0.35 (87↓)		5.8 (6.4↓)	4	9(25↑)	5.25 (25↑)	5.88	2.97	5.2 (26.8↑)		5.8	1.1 (32.5↑)	0.07 (80.6↓)
carob seed germ meal	34.8	6.49 (13.5↓)	1.41 (47.8↓)		14.1 (127↑)		7.1	3.88 (7.6↓)	3.64	3.5	4.14(1↑)		4.31	2.17 (161.4↑)	0.22 (38.9↓)

PC: protein concentrate; PI: Protein Isolate; Lys: lysine; Met: methionine; Cys: cysteine; Arg: arginine; Trp: tryptophan; Leu: leucine; Ile: Isoleucine; Phe: phenylalanine; His: histidine; Thr: threonine; Tyr: tyrosine; Val: valine.

Table 2.
Amino acid content of legumes products in comparison to fish meal.

2.5 Soy protein hydrolysates

Soy protein hydrolysate is produced by restricted enzymatic hydrolysis of soy products that consequently improves their nutritional and practical characteristics [30]. The enzymes generally use for hydrolysis of soy protein are mainly endo and exopeptidases (*e.g.* leucine aminopeptidase) that derived from fermentation of selected strains of bacteria (*e.g. Bacillus licheniformis*) [30]. The most important characteristics of SPH are the maximized digestibility of protein, excellent protein solubility and minimized ANF that ultimately enhance its efficiency. Significant solubility of the SPH is mainly due to the formation of short chain hydrophilic polypeptides and the elimination of insoluble fractions through a sedimentation step by a centrifugation process [31]. In addition, SPH has numerous bioactive low molecular weights peptides with health improving properties such as antioxidative and immunostimulatory compounds [32–34].

2.6 Fermented soybean meal

The fermentation process in aquaculture usually uses for enhancing the nutritional value and reducing ANF in alternative protein sources for incorporating into aquafeeds [35]. Fermentation of SBM by yeast (*e.g. Saccharomyces cerevisiae*), fungus (*e.g. Aspergillus niger*) or bacterial strains (*e.g. Bacillus spp., L. plantarum P8, Pediococcus acidilacticstrains*) can improve its nutritional quality and digestibility by providing low molecular weight peptides, increasing bioavailability of minerals and reducing its ANF (*e.g. trypsin inhibitors*) [35–37]. Moreover, fermented SBM has more protein content (~10%) than SBM with negligible change of its EAA profile [37]. In addition, fermented SBM provide probiotic characteristics and can increase efficiency of aquafeeds by elevating trypsin and fibrinolytic enzymes activities [35].

2.7 Transgenic soybean

"Genetically modified plants are typically created by the addition or deletion of existing innate genes in the plant's own genome or transferring external non-host genome through DNA splicing" [38]. Genetic approaches were applied for creating a prototype soybean that synthetize and accumulate a n-3 long chain polyunsaturated fatty acid (*i.e.* eicosapentaenoic acid, EPA) and a carotenoid (*i.e.* astaxanthin) in the seed [15]. Soybean contains very low levels of lutein (10 µg/g seed), but the expression of the phytoene synthase gene in transgenic soybean increases the accumulation of ß-carotene up to 800 µg/g seed and significantly reduces lutein content (~29%). The expression of fatty acid elongases and Δ5 desaturase in transgenic soybean increase the synthesis of EPA up to 5%, but EPA content need to be improve in transgenic soybean to better reflect FO fatty acid profile [15]. It should be mentioned that the annual sales of astaxanthin reaches to over USD 200 million and inclusion of such trait in transgenic soybean can impressively enhance the attractiveness of such product especially for incorporating in aquafeeds for salmon, trout and shrimp [39, 40].

2.8 Pea protein

Pea (*Pisum sativum*) is another promising APPS for aquaculture species with highly digestible protein and energy levels and it is a good source of digestible starch (~40-50%) [41, 42]. This legume contains low levels of ANF (*e.g.* tannins) and does not have trypsin inhibitors, but contain high levels of saponins. The protein (~21-25%) and methionine contents in peas also lower compared to soybean. Pea protein concentrate (PPC) is a pea derived product that produced by fine grinding dehulled peas and air processing to remove fiber and carbohydrates. The PPC contains higher protein and lower ANF compared to unprocessed pea meal, thus it is more suitable APPS for aquafeeds. Like other plant protein derived products, extrusion and micronizing processes improve protein and energy digestibility of pea meals [41].

2.9 Peanut

Peanut (*Arachis hypogaea*) is the fourth largest oilseed crop in the world and peanut pulp that remain after the oil extraction can be used as an APPS in aquafeeds [43, 44]. Peanut meal (PNM) is a residue after solvent extraction of whole shelled peanuts and considered as a great APPS due to its higher protein content (~47.8%) than SBM, higher palatability and the same cost as SBM [45, 46]. However, the protein quality of PNM is inferior compared to SBM and it contains lower levels of lysine and methionine than SBM, but a higher level of arginine [43, 45]. Lysine and methionine deficiencies in PNM can be met by using crystalline amino acids.

2.10 Lupines

Lupines include many legumes species with a considerable protein (~ 35%) but low lipid (~8–10%) levels [21, 47]. About 80% of lupines species the can be used as feed ingredients, particularly *Lupinus angustifolius,* is produced in Australia. Among different lupines, Andean lupin (*L. mutabilis*) seed contain ~50% protein (dry matter) and its derivatives such as dehulled, deoiled and lupine protein concentrate contain higher protein content (~61%) [48]. Although lupines have low levels of lysine and sulfur amino acids, they contain more arginine content than soybean [49]. Four species of lupines including *L. angustifolius, L. albus, L. luteus and L. mutabilis* named as "sweet lupins" as they contains low levels of alkaloids and because of their high protein contents they have great potential as APPS [50]. However, using lupins in aquafeeds are still limited because of their low protein digestibility and the presence of various ANF [51].

2.11 Faba bean

Faba bean, (*Vicia faba* L.) is a legume with high amount of protein (~ 20 to 41%), carbohydrate (~51% to 68%), B-vitamins and minerals depending on its variety [52, 53]. Its protein composition is mainly consisted of albumins (20%) and globulins (80%) and rich in glutamic and aspartic acids. But, the levels of sulfur amino acids and tryptophan residues are low [54]. The main carbohydrates in faba been are starch (~41–53%), low molecular weight carbohydrates (*e.g.* raffinose , stachyose, and verbascose), and fiber mainly hemicellulose [52, 55]. Faba bean contains some ANF such as trypsin inhibitors and lectins, condensed tannin, phytic acid, vicine and convicine [56]. Processing of faba bean protein does provide ingredients with higher protein contents such as faba bean protein concentrate (~55% crude protein) and faba bean isolate (~80% crude protein) that contain lower levels of ANF [57–59].

2.12 Other protein sources

Carob seed (*Ceratonia siliqua*) germ does have a high protein content (~45–50% crude protein) and it is cheaper than SBM [60]. Carob seed germ meal is produced from the germ of the carob seed after the separation of the gums and the fibrous [61–63]. However, it contains high levels of tannins [64].

Alfalfa (*Medicago sativa*) protein concentrate is another APPS that produced by pressing fresh alfalfa foliage (mainly leaves and stems) to make a protein-rich juice which is centrifuged and heated to fractionate proteins from the juice [65]. This byproduct contains reasonable protein level (~52% crude protein) with high amounts of lysine, threonine, and methionine. It also contains high levels of vitamins and antioxidants such as carotenoids, but low content of fiber and ANF (*e.g.* phytic acid or lectins) [65, 66].

3. Anti-nutritional factors in legumes

The ANF are defined as compounds that disturb feed utilization and can affect the health condition and production of livestock [67]. Legumes contain various ANF such as saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, antivitamin factors, metal binding ingredients, goitrogens, etc. (**Table 1**) that combine with nutrients and reduce bioavailability of them in aquafeeds [8]. Some ANF such as protease inhibitors and phytates abate digestibility of proteins and energy as well as reduce mineral absorption that consequently results in malnutrition and microelements deficiencies.

The ANF can be divided into four classes [66]:

I. Substances that affect dietary protein utilization (*e.g.* protease inhibitors, tannins and lectins).

II. Substances that influence dietary mineral utilization (*e.g.* phytates, gossypol, oxalates and glucosinolates)

III. Antivitamins

IV. Miscellaneous (*e.g.* non-starch polysaccharides, mycotoxins, alkaloids, pyrimidine glycosides, phytoestrogens and saponins).

4. Improvement of legumes efficiency in aquafeeds

Digestibility of an ingredient is a pivotal parameter for determining its potential for use in the aquafeeds [68]. In order to validate the nutritional quality of a feedstuff, determination of apparent digestibility coefficient (ADC) of its dry matter and nutrients is necessary. As previously mentioned, generally legumes contain high amounts of starch and NSP and ANF [66] that negatively affect ADC in most fish and shrimp species. Carnivorous fish species are more susceptible to legumes. The ADC of crude protein of legumes are generally over 0.80, indicating high quality of protein provided by these APS. However, the ADC of gross energy in these research showed great fluctuations from 0.5 to 0.7 [69]. It has been reported that the ADC of legumes in diet mainly depends on fish species. Thus, ADC of legumes in omnivorous species such as Nile tilapia is higher than carnivorous fish such as rainbow trout [69].

Several strategies were applied for improving nutrients digestibility in legumes such as processing techniques (*e.g.* dehulling, soaking, extrusion cooking, fermentation etc.), using novel and new variety of plant protein sources (*e.g.* transgenic legumes), nutritional programming and selective breeding of fish to be more adapted to legumes in aquafeeds, modulation of gut microbiota (*e.g.* probiotics and short chain fatty acids), and inclusion of additives (*e.g.* acidifiers, CAA, phospholipids etc.) in aquafeeds [53, 70]. Here the most efficient strategies for reducing ANF in APPS were described:

4.1 Conventional strategies

Several physical processing strategies applied for removing, inactivating or reducing ANF (*e.g.* trypsin inhibitors, glucosinolates, tannins and saponins) contents in APPS including heat and/or soaking in water, dehulling and germination, roasting or autoclaving as well as extrusion and micronizing (infrared heat) [71] (**Table 3**). These conventional methods positively improve digestibility of legumes; however, these strategies are not conclusive in eradicating the adverse influences of ANF in legumes [72]. Moreover, some strategies such as heat damage lead to loss of some amino acids and adversely affect quality of proteins and carbohydrates through Malliard reactions [73, 74]. Furthermore, soaking in water may result in leaching water-soluble nutrients by this process.

4.2 Exogenous enzymes and phytase

It has been confirmed that inclusion of carbohydrase exogenous enzymes such as xylanae, ß-glucanase and cellulase as well as phytase in aquafeeds can reduce the negative effects of NSP and phytate on digestion [75, 76]. Exogenous carbohydrases by facilitating carbohydrate digestion and reducing feed polymerization degree is going to decrease its viscosity and liberate carbohydrate oligomers [77]. In addition, carbohydrases by neutralizing NSP can increase the digestibility of energy, macronutrients and bioavailability of minerals because NSP reduce accessibility of enzymes to substrates and there is a relationship between phytate and NSP in PPS [75, 78]. In addition, carbohydrases may improve host's gut health by supporting the propagation of beneficial microbiota in the gut that can facilitate fermentation of NSP and consequently increase the amounts of organic acids and especially short chain fatty acids production [78, 79].

4.3 Acidifiers

A plethora of studies confirmed that high amounts of dietary FM could be substituted with APPS by supplementing diet with short-chain fatty acids and acidifiers [80, 81]. In fact, acidification of plant protein based aquafeeds with acidifiers increase the bioavailability of minerals and trace elements and they neutralize or alleviate the negative impacts of ANF on nutrients digestibility [60, 82–84]. In addition, acidifiers by reducing the chyme pH through the gut can induce the pepsin activity [85]. Moreover, reduction of the chyme pH triggers the release of gastrointestinal hormones (*e.g.* secretin and cholecystokinin) that stimulate secretion of pancreatic digestive enzymes, which in turn elevates the digestibility of protein and minerals. Furthermore, it has been reported that acidifiers by controlling the appetite through the parasympathetic nerve system including orexigenic neurotransmitters that increase feed efficiency [86].

4.4 Gut microbiota as ANF biodegrading agent in fish

The application of the gut "indigenous" microbiota as probiotics can improve feed digestibility by supplying exogenous enzymes (*e.g.* cellulase, phytase, tannase and xylanases) and by eradicating and/or reducing ANF of the plant protein

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Reduction strategies	**Bioactive component**															
	Protease/trypsin inhibitors	**Amylase inhibitors**	**Saponins**	**Phytate**	**Glucosinolates**	**Oxalate**	**Anti-vitamins**	**Polyphenols/Tannins**	**Sterols**	**Lectins**	**Phytoestrogens**	**Alkaloids**	**Cyanogens**	**NSP**	**Oligosacharide**	**Antigenic proteins**
Milling				●				●		●						
De-hulling								●				●				
Soaking	●			●				●			●	●				●
Heat/Autoclaved	●	●	●	●	●	●	●	●		●	●					●
Solvent extraction			●	●			●		●		●	●			●	●
Germination	●			●				●								●
Acid addition	●		●	●			●							●	●	
Alkalin addition							●	●				●		●	●	●
enzymes addition	●	●		●										●		●
Fermentation	●		●	●		●		●			●	●	●	●	●	●
Micronutrient addition	●			●					●	●			●			
New Variates				●	●							●				

Table 3.
Legume bioactive compounds neutralizing strategies.

ingredients in the fish gut [87]. A plethora of studies have recognized cellulase-producing bacteria such as *Citrobacter sp. C. freundii*, *Enterobacter* sp. *Bacillus coagulans*, *B. cereus*, *B. subtilis* P6, *B. velesensis* P11, *B. pumilus*, *B. tequilensis* (KF640219), *B. megaterium* (KF640220) and *B. altitudinis* from the gut of various cultured fish species including Chinese carps [88, 89], Indian major carps [90, 91], tilapia (*Oreochromis mossambica*) [92], bata fish [93], murrels (*Channa punctatus*) [94], pacu (*Piaractus mesopotamicus*) and piaucom-pinta (*Leporinus friderici*) [95]. Using the above mentioned microorganisms as potential probiotics in aquafeeds or applying these microorganisms for fermentation of plant protein ingredients can provide great potential for eradicating ANF and improving their nutritional quality by boosting up EAA, minerals and vitamins bioavailability and increasing digestibility of protein and energy.

4.5 Other functional feed additives

Supplementing PP-based aquafeeds with additives can improve the digestibility of feed's nutrients. In this context, it has been reported that supplementation of a diet contained high levels of legumes including SPC and pea protein (32%) with phosphatidylcholine pronouncedly improved lipid digestibility in Atlantic salmon [96].

On the other hand, it has been confirmed that the replacement of FM with PP sources could reduce cholesterol content in aquafeeds that may disturb bile acids synthesis in fish and result in low digestibility of lipid [97]. In this regard, it has been reported that supplementing SBM-based diets with cholesterol remarkably improved growth in channel catfish [98], turbot (*Scophthalmus maximus*) [85] and rainbow trout [99].

As mentioned earlier legumes are deficient in taurine or its precursors (*i.e.* cysteine and methionine) and some aquatic animal species especially marine fish unable, or have low ability to synthesize taurine [100]. Taurine is the main component of bile acids and increase the bile-salt dependent lipase activity in fish [101]. It has been proved that supplementation of soy protein-based aquafeeds with taurine improved growth performance, lipid metabolism, palatability, digestibility and overall nutritional quality of feeds in marine fish species such as common dentex (*Dentex dentex*) [101] and European sea bass larvae [102] and juveniles [103, 104].

Moreover, it has been confirmed that replacement of FM with plant protein sources with high levels of ANF (*i.e.* saponins, oligosaccharides, fibers and high molecular weight proteins) disturb bile metabolism in fish and may adversely affect fish productivity [105]. Bile acids as an emulsifier enhance digestion and absorption of lipid and lipid soluble nutrients through emulsification of lipids and activation of bile salt dependent lipase [105]. It also facilitates the excretion of cholesterol and toxic metabolites. The ANF in PP sources may induce gut inflammation that reduce resorption of bile acids or they may bind with bile salts and trigger extra excretion of bile acids into gut [105]. Thus, supplementing legume protein-based diet with bile acids may improve their efficiency and alleviate their negative effects on fish performance. For example, supplementing SBM-based diet with 1.5% bovine bile salts significantly improved growth rate in rainbow trout [106].

4.6 Nutritional programming and selective breeding

In recent years some studies were carried out on early nutritional programming of fish for increasing the acceptance of their offspring to new ingredients in aquafeeds. For instance, it has been reported that substitution of dietary FM and FO with vegetal feedstuffs through nutritional programming in brooders elevated the acceptance of vegetal ingredients and PP-based diets in rainbow trout [107] and gilthead seabream [108] offspring. In this regard, it has been reported that early nutritional programming in Atlantic salmon with a plant-based aquafeed enhanced growth rate and feed efficiency for 24% and 23%, respectively compared to those fed a diet contained FM and FO and then challenged with a plant-based aquafeed [109].

Recently, a new strain of rainbow trout (ARS-KO) was created by the US department of Agriculture by selective breeding over the course of two decades and this strain can grow better when fed with soy protein-based diets and does not develop enteritis [110, 111]. More research are required to be carried out in these genetic engineering to these novel techniques be advantageous and applicable at commercial stage.

5. Conclusions

Over the course of the past four decades, a great amount of knowledge has been gained in application of legumes as APS in aquafeeds, leading to a better comprehension regarding the impacts of these APPS on overall performance of different cultured species. Herbivorous and omnivorous fish and crustacean species have a great potential in utilization of legumes in their diets. Moreover, carnivorous species have mostly adapted to legumes-protein rich aquafeeds. However, in order to enhance the efficiency of legumes-protein based aquafeeds for carnivorous fish, further innovations and development is required by considering the cultured animal species and feed ingredients for increasing adaptability of cultured aquatic species to legumes. These innovations can be carried out in different aspects such as use of novel feedstuffs, eradication and/or reducing ANF, application of feed additives and use of precise feed formulations. The application of genetic engineering in legumes could result in the production of strains with low levels of ANF and make them appropriate for legumes-protein based aquafeeds. In addition, supplementing aquafeeds with functional feed additives can improve the efficiency of legumes for aquaculture nutrition. Using nutritional programming and applying genetic engineering also other novel strategies to provide new fish and crustacean strains with high capacity in acceptance and utilization of legumes in aquafeeds. Further studies are required to increase the efficiency of legumes in aquafeeds to support growth, health and welfare of cultured species.

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