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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References

[1] Markets, Markets . https://wwwmarketsandmarketscom/new-reportshtml. 2021.

[2] FAO. Sustainability in action. State of World Fisheries and Aquaculture Rome. 2020;200.

[3] FAO. The State of World Fisheries and Aquaculture 2018—Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO. 2018.

[4] Henry M, Gasco L, Piccolo G, Fountoulaki E. Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology. 2015;203:1-22. DOI: 10.1016/j.anifeedsci.2015.03.001.

[5] Chakraborty P, Mallik A, Sarang N, Lingam SS. A review on alternative plant protein sources available for future sustainable aqua feed production. Int J Chem Stud. 2019;7:1399-404.

[6] Hardy RW. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research. 2010;41(5): 770-6. DOI: 10.1111/j.1365-2109.2009.02349.x.

[7] Tacon AG, Metian M. Feed matters: satisfying the feed demand of aquaculture. Reviews in Fisheries Science & Aquaculture. 2015;23(1):1-10. 10.1080/23308249.2014.987209.

[8] Gatlin III DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, et al. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture research. 2007;38 (6):551-79. 10.1111/j.1365-2109.2007.01704.x.

[9] Tacon AG, Metian M. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 2008;285(1-4):146-58. DOI: 10.1016/j.aquaculture.2008.08.015.

[10] Tacon AG, Hasan MR, Metian M. Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects. FAO Fisheries and Aquaculture technical paper. 2011(564):I.

[11] Oliva-Teles A, Enes P, Peres H. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed and feeding practices in aquaculture. 2015:203-33. 10.1016/B978-0-08-100506-4.00008-8.

[12] McNeill J, Barrie F, Buck W, Demoulin V, Greuter W, Hawksworth D, et al. International Code of Nomenclature for algae, fungi and plants (Melbourne Code): Koeltz Scientific Books Königstein; 2012.

[13] Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, et al. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017: Koeltz Botanical Books; 2018.

[14] Faostat F. Crops. Food and Agriculture Organization of the United Nations Available online at. 2020.

[15] Park H, Weier S, Razvi F, Peña PA, Sims NA, Lowell J, et al. Towards the development of a sustainable soya bean-based feedstock for aquaculture. Plant biotechnology journal. 2017;15(2): 227-36. 10.1111/pbi.12608.

[16] Liu K. Chemistry and nutritional value of soybean components. Soybeans: Springer; 1997. p. 25-113.

[17] Refstie S, Svihus B, Shearer KD, Storebakken T. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch

polysaccharide content in different soyabean products. Animal Feed Science and Technology. 1999;79(4):331-45. DOI: 10.1016/S0377-8401(99)00026-7.

[18] Elangovan A, Shim K. The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (Barbodes altus). Aquaculture. 2000;189(1-2):133-44. 10.1016/S0044-8486(00)00365-3.

[19] Salze G, McLean E, Battle PR, Schwarz MH, Craig SR. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, Rachycentron canadum. Aquaculture. 2010;298(3-4):294-9. DOI: 10.1016/j.aquaculture.2009.11.003.

[20] Mamauag R, Koshio S, Ishikawa M, Yokoyama S, Gao J, Nguyen B, et al. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder, Paralichthys olivaceus. Aquaculture. 2011;321(3-4):252-8. 10.1016/j.aquaculture.2011.09.022.

[21] Hartviksen MAB. Replacement of fishmeal with alternative proteins in diets for Atlantic salmon (Salmo salar L.): A study on the microbiota, morphology and function of the intestine 2015.

[22] Bureau DP, Harris AM, Cho CY. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (Oncorhynchus mykiss). Aquaculture. 1998;161(1-4):27-43. DOI: 10.1016/S0044-8486(97)00254-8.

[23] Storebakken T, Shearer K, Roem A. Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmon Salmo salar fed diets with fish meal and soy-protein concentrate as the main sources of protein. Aquaculture Nutrition. 2000;6(2):103-8.

[24] Storebakken T, Shearer K, Roem A. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, Salmo salar. Aquaculture. 1998;161(1-4):365-79. DOI: 10.1016/S0044-8486(97)00284-6.

[25] Gamboa-Delgado J, Rojas-Casas MG, Nieto-López MG, Cruz-Suárez LE. Simultaneous estimation of the nutritional contribution of fish meal, soy protein isolate and corn gluten to the growth of Pacific white shrimp (Litopenaeus vannamei) using dual stable isotope analysis. Aquaculture. 2013;380:33-40. DOI: 10.1016/j.aquaculture.2012.11.028.

[26] Alam MS, Teshima S-i, Koshio S, Ishikawa M, Uyan O, Hernandez LHH, et al. Supplemental effects of coated methionine and/or lysine to soy protein isolate diet for juvenile kuruma shrimp, Marsupenaeus japonicus. Aquaculture. 2005;248(1-4):13-9. DOI: 10.1016/j.aquaculture.2005.04.015.

[27] Ireland PA, Dziedzic SZ. Effect of hydrolysis on sapogenin release in soy. Journal of Agricultural and Food Chemistry. 1986;34(6):1037-41. DOI: 10.1021/jf00072a027.

[28] Anderson RL, Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. The Journal of nutrition. 1995;125(suppl_3):581S-8S. DOI: 10.1093/jn/125.suppl_3.581S.

[29] Glencross B, Evans D, Dods K, McCafferty P, Hawkins W, Maas R, et al. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to

rainbow trout, Oncorhynchus mykiss, using either stripping or settlement faecal collection methods. Aquaculture. 2005;245(1-4):211-20. 10.1016/j. aquaculture.2004.11.033.

[30] Hrckova M, Rusnakova M, Zemanovic J. Enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on the functional properties of resulting protein hydrolysates. Czech journal of food sciences. 2002;20(1):7-14.

[31] Kristinsson HG, Rasco BA. Biochemical and functional properties of Atlantic salmon (Salmo salar) muscle proteins hydrolyzed with various alkaline proteases. Journal of agricultural and food chemistry. 2000; 48(3):657-66. DOI: 10.1021/jf990447v.

[32] Chen H-M, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. Journal of agricultural and food chemistry. 1998; 46(1):49-53. 10.1021/jf970649w.

[33] Peñta-Ramos E, Xiong Y. Antioxidant activity of soy protein hydrolysates in a liposomal system. Journal of food science. 2002;67(8):2952-6.

[34] Maruyama N, Fukuda T, Saka S, Inui N, Kotoh J, Miyagawa M, et al. Molecular and structural analysis of electrophoretic variants of soybean seed storage proteins. Phytochemistry. 2003; 64(3):701-8. DOI: 10.1016/S0031-9422 (03)00385-6.

[35] Dawood MA, Koshio S. Application of fermentation strategy in aquafeed for sustainable aquaculture. Reviews in Aquaculture. 2020;12(2):987-1002. 10.1111/raq.12368.

[36] Kim B. Physiological functions of chongkukjang. Food Industry and Nutrition. 1999;4:40-6.

[37] Hong K-J, Lee C-H, Kim SW. Aspergillus oryzae GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. Journal of medicinal food. 2004;7(4): 430-5. DOI: 10.1089/jmf.2004.7.430.

[38] Osmond AT, Colombo SM. The future of genetic engineering to provide essential dietary nutrients and improve growth performance in aquaculture: Advantages and challenges. Journal of the World Aquaculture Society. 2019;50 (3):490-509. 10.1111/jwas.12595.

[39] Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea F. Astaxanthin: a review of its chemistry and applications. Critical reviews in food science and nutrition. 2006;46(2):185-96. 10.1080/ 10408690590957188.

[40] Hussein G, Sankawa U, Goto H, Matsumoto K, Watanabe H. Astaxanthin, a carotenoid with potential in human health and nutrition. Journal of natural products. 2006;69(3):443-9. 10.1021/np050354+.

[41] Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, McCallum IM, Hickling D. Assessment of differently processed feed pea (Pisum sativum) meals and canola meal (Brassica sp.) in diets for blue shrimp (Litopenaeus stylirostris). Aquaculture. 2001;196 (1-2):87-104. 10.1016/S0044-8486(00) 00572-X.

[42] Hickling D. Canadian feed peas industry guide. Pulse Canada, Winnipeg, Manitoba. 2003:1-36.

[43] Liu Xh, Ye Jd, Wang K, Kong Jh, Yang W, Zhou L. Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, Litopenaeus vannamei. Aquaculture Research. 2012;43(5):745-55. 10.1111/ j.1365-2109.2011.02883.x.

[44] Yıldırım Ö, Acar Ü, Türker A, Sunar MC, Kesbic OS. Effects of

Replacing Fish Meal with Peanut Meal (Arachis hypogaea) on Growth, Feed Utilization and Body Composition of Mozambique Tilapia Fries (Oreochromis mossambicus). Pakistan Journal of Zoology. 2014;46(2):497-502.

[45] Batal A, Dale N, Café M. Nutrient composition of peanut meal. Journal of applied poultry research. 2005;14(2): 254-7. DOI: 10.1093/japr/14.2.254.

[46] Jiang H, Qi P, Wang T, Chi X, Wang M, Chen M, et al. Role of halotolerant phosphate-solubilising bacteria on growth promotion of peanut (Arachis hypogaea) under saline soil. Annals of Applied Biology. 2019;174(1): 20-30. DOI: 10.1111/aab.12473.

[47] Draganovic V. Towards sustainable fish feed production using novel protein sources. Wageningen: Wageningen University; 2013.

[48] Molina-Poveda C, Lucas M, Jover M. Evaluation of the potential of Andean lupin meal (Lupinus mutabilis Sweet) as an alternative to fish meal in juvenile Litopenaeus vannamei diets. Aquaculture. 2013;410:148-56. 10.1016/ j.aquaculture.2013.06.007.

[49] Glencross BD. Feeding lupins to fish: A review of the nutritional and biological value of lupins in aquaculture feeds: Department of Fisheries– Research Division, Government of Western Australia; 2001.

[50] Drew M, Borgeson T, Thiessen D. A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology. 2007;138(2):118-36. DOI: 10.1016/j. anifeedsci.2007.06.019.

[51] Estensoro I, Ballester-Lozano G, Benedito-Palos L, Grammes F, Martos-Sitcha JA, Mydland L-T, et al. Dietary butyrate helps to restore the intestinal status of a marine teleost (Sparus aurata) fed extreme diets low in fish meal and fish oil. PLoS One. 2016;11 (11):e0166564. DOI: 10.1371/journal. pone.0166564.

[52] Revilla I. Impact of thermal processing on faba bean (Vicia faba) composition. Processing and impact on active components in food: Elsevier; 2015. p. 337-43.

[53] Alinasabhematabadi L. Evaluation of apparent nutrient digestibility in Atlantic salmon fed diets with differently processed faba bean: Norwegian University of Life Sciences, Ås; 2019.

[54] Multari S, Stewart D, Russell WR. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety. 2015;14(5):511-22. DOI: 10.1111/ 1541-4337.12146.

[55] Vidal-Valverde C, Frias J, Sotomayor C, Diaz-Pollan C, Fernandez M, Urbano G. Nutrients and antinutritional factors in faba beans as affected by processing. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 1998;207(2):140-5. DOI: 10.1007/s002170050308.

[56] Helsper J, Van Norel A. Antinutritional Factors in Faba Bean and Resistance to Soil-Borne Diseases. Developments in Agricultural and Managed Forest Ecology. 23: Elsevier; 1991. p. 387-91.

[57] De Santis C, Crampton VO, Bicskei B, Tocher DR. Replacement of dietary soy-with air classified faba bean protein concentrate alters the hepatic transcriptome in Atlantic salmon (Salmo salar) parr. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics. 2015;16: 48-58. 10.1016/j.cbd.2015.07.005.

[58] De Santis C, Ruohonen K, Tocher DR, Martin SA, Krol E,

Secombes CJ, et al. Atlantic salmon (Salmo salar) parr as a model to predict the optimum inclusion of air classified faba bean protein concentrate in feeds for seawater salmon. Aquaculture. 2015;444: 70-8. 10.1016/j.aquaculture.2015.03.024.

[59] Król E, Douglas A, Tocher DR, Crampton VO, Speakman JR, Secombes CJ, et al. Differential responses of the gut transcriptome to plant protein diets in farmed Atlantic salmon. BMC genomics. 2016;17(1): 1-16. DOI: 10.1186/s12864-016-2473-0.

[60] Hossain MA, Pandey A, Satoh S. Effects of organic acids on growth and phosphorus utilization in red sea bream Pagrus major. Fisheries Science. 2007;73 (6):1309-17. DOI: 10.1111/ j.1444-2906.2007.01469.x.

[61] Alexis MN, Papaparaskeva-Papoutsoglou E, Theochari V. Formulation of practical diets for rainbow trout (Salmo gairdneri) made by partial or complete substitution of fish meal by poultry by-products and certain plant by-products. Aquaculture. 1985;50(1-2):61-73. DOI: 10.1016/ 0044-8486(85)90153-X.

[62] Martínez-Llorens S, Baeza-Ariño R, Nogales-Mérida S, Jover-Cerdá M, Tomás-Vidal A. Carob seed germ meal as a partial substitute in gilthead sea bream (Sparus aurata) diets: amino acid retention, digestibility, gut and liver histology. Aquaculture2012. p. 124-33.

[63] Couto A, Barroso C, Guerreiro I, Pousão-Ferreira P, Matos E, Peres H, et al. Carob seed germ meal in diets for meagre (Argyrosomus regius) juveniles: Growth, digestive enzymes, intermediary metabolism, liver and gut histology. Aquaculture. 2016;451: 396-404. DOI: 10.1016/j. aquaculture.2015.10.007.

[64] Avallone R, Plessi M, Baraldi M, Monzani A. Determination of chemical composition of carob (Ceratonia siliqua): protein, fat, carbohydrates, and tannins. Journal of food composition and analysis. 1997;10(2):166-72. DOI: 10.1006/jfca.1997.0528.

[65] Koegel R, Straub R. Fractionation of alfalfa for food, feed, biomass, and enzymes. Transactions of the ASAE. 1996;39(3):769-74. 10.13031/2013.27559.

[66] Francis G, Makkar HP, Becker K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 2001;199(3-4):197-227. 10.1016/ S0044-8486(01)00526-9.

[67] Makkar H. Antinutritional factors in foods for livestock. BSAP Occasional Publication. 1993;16:69-85. DOI: 10.1017/S0263967X00031086.

[68] Allan GL, Parkinson S, Booth MA, Stone DA, Rowland SJ, Frances J, et al. Replacement of fish meal in diets for Australian silver perch, Bidyanus bidyanus: I. Digestibility of alternative ingredients. Aquaculture. 2000;186 (3-4):293-310. 10.1016/S0044-8486(99) 00380-4.

[69] Magalhaes S, Cabrita A, Valentao P, Andrade P, Rema P, Maia M, et al. Apparent digestibility coefficients of European grain legumes in rainbow trout (Oncorhynchus mykiss) and Nile tilapia (Oreochromis niloticus). Aquaculture nutrition. 2018;24(1): 332-40. DOI: 10.1111/anu.12564.

[70] Colombo SM. Physiological considerations in shifting carnivorous fishes to plant-based diets. In: Benfey TJ, Farrell AP, Brauner CJ, editors. Fish Physiol. 38: Academic Press; 2020. p. 53-82.

[71] Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Production, Processing and Nutrition. 2020;2(1):1-14. DOI: 10.1186/ s43014-020-0020-5.

[72] Mohanta K. Plant feed resources of India. In: Sogbesan OA, Mohanta KN, Sahoo PK, Mitra G, Jayasankar P, editors. Invited Papers on Application of Solid State Fermentation Technology in Aquaculture. Central Institute of Freshwater Aquaculture, Bhubaneswar, India2012. p. 100-13.

[73] Glencross B, Hawkins W, Curnow J. Nutritional assessment of Australian canola meals. I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red seabream (Pagrus auratus, Paulin). Aquaculture Research. 2004;35(1):15-24. DOI: 10.1111/j.1365-2109.2004.00974.x.

[74] Glencross B, Hawkins W, Curnow J. Nutritional assessment of Australian canola meals. II. Evaluation of the influence of the canola oil extraction method on the protein value of canola meals fed to the red seabream (Pagrus auratus, Paulin). Aquaculture Research. 2004;35(1):25-34. DOI: 10.1111/j.1365-2109.2004.00975.x.

[75] Castillo S, Gatlin III DM. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. Aquaculture. 2015;435:286-92. DOI: 10.1016/j.aquaculture.2014.10.011.

[76] Maas RM, Verdegem MC, Dersjant-Li Y, Schrama JW. The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. Aquaculture. 2018;487:7-14. DOI: 10.1016/j.aquaculture.2017.12.040.

[77] Vahjen W, Osswald T, Schäfer K, Simon O. Comparison of a xylanase and a complex of non starch polysaccharide-degrading enzymes with regard to performance and bacterial metabolism in weaned piglets. Archives of animal nutrition. 2007;61(2):90-102. DOI: 10.1080/17450390701203881.

[78] Adeola O, Cowieson A. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Journal of animal science. 2011;89(10):3189-218. DOI: 10.2527/jas.2010-3715.

[79] Kiarie E, Nyachoti C, Slominski B, Blank G. Growth performance, gastrointestinal microbial activity, and nutrient digestibility in early-weaned pigs fed diets containing flaxseed and carbohydrase enzyme. Journal of Animal Science. 2007;85(11):2982-93. DOI: 10.2527/jas.2006-481.

[80] Ng WK, Koh CB. The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Reviews in Aquaculture. 2017;9(4):342-68. DOI: 10.1111/raq.12141.

[81] Sarker M, Satoh S, Kamata K, Haga Y, Yamamoto Y. Partial replacement of fish meal with plant protein sources using organic acids to practical diets for juvenile yellowtail, Seriola quinqueradiata. Aquaculture Nutrition. 2012;18(1):81-9. DOI: 10.1111/j.1365-2095.2011.00880.x.

[82] Khajepour F, Hosseini SA. Citric acid improves growth performance and phosphorus digestibility in Beluga (Huso huso) fed diets where soybean meal partly replaced fish meal. Animal feed science and technology. 2012;171(1):68-73. DOI: 10.1016/j.anifeedsci.2011.10.001.

[83] Lin Y-H, Cheng M-Y. Effects of dietary organic acid supplementation on the growth, nutrient digestibility and intestinal histology of the giant grouper Epinephelus lanceolatus fed a diet with soybean meal. Aquaculture. 2017;469:106-11. DOI: 10.1016/j.aquaculture.2016.11.032.

[84] Sarker MSA, Satoh S, Kamata K, Haga Y, Yamamoto Y. Supplementation effect (s) of organic acids and/or lipid to plant protein-based diets on juvenile yellowtail, Seriola quinqueradiata

Temminck et Schlegel 1845, growth and, nitrogen and phosphorus excretion. Aquaculture Research. 2012;43(4): 538-45. DOI: 10.1111/j.1365-2109.2011. 02859.x.

[85] Castillo S, Rosales M, Pohlenz C, Gatlin III DM. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum Sciaenops ocellatus. Aquaculture. 2014; 433:6-12. DOI: 10.1016/j.aquaculture. 2014.05.038.

[86] Zhang J, Zhong L, Chi S, Chu W, Liu Y, Hu Y. Sodium butyrate supplementation in high-soybean meal diets for juvenile rice field eel (Monopterus albus): Effects on growth, immune response and intestinal health. Aquaculture. 2020;520:734952. 10.1016/ j.aquaculture.2020.734952.

[87] Ghosh K, Ray AK. Aquafeed formulation using plant feedstuffs: Prospective application of fish-gut microorganisms and microbial biotechnology. Soft Chemistry and Food Fermentation: Elsevier; 2017. p. 109-44.

[88] Bairagi A, Ghosh KS, Sen SK, Ray AK. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International. 2002; 10(2):109-21. DOI: 10.1023/A: 1021355406412.

[89] Li J, Tan B, Mai K. Dietary probiotic Bacillus OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (Litopenaeus vannamei). Aquaculture. 2009;291(1-2):35-40. DOI: 10.1016/j.aquaculture.2009.03.005.

[90] Ray AK, Roy T, Mondal S, Ringø E. Identification of gut-associated amylase, cellulase and protease-producing bacteria in three species of Indian major carps. Aquaculture Research. 2010;41 (10):1462-9. DOI: 10.1111/j.1365-2109. 2009.02437.x.

[91] Banerjee S, Mukherjee A, Dutta D, Ghosh K. Non-Starch Polysaccharide Degrading Gut Bacteria in Indian Major Carps and Exotic Carps. Jordan Journal of Biological Sciences. 2016;9(1). DOI: 10.12816/0027010.

[92] Saha S, Roy RN, Sen SK, Ray AK. Characterization of cellulase-producing bacteria from the digestive tract of tilapia, Oreochromis mossambica (Peters) and grass carp, Ctenopharyngodon idella (Valenciennes). Aquaculture Research. 2006;37(4):380-8. DOI: 10.1111/ j.1365-2109.2006.01442.x.

[93] Mondal S, Roy T, Ray AK. Characterization and identification of enzyme-producing bacteria isolated from the digestive tract of bata, Labeo bata. Journal of the World Aquaculture Society. 2010;41(3):369-77.

[94] Kar N, Ghosh K. Enzyme producing bacteria in the gastrointestinal tracts of Labeo rohita (Hamilton) and Channa punctatus (Bloch). Turkish Journal of Fisheries and Aquatic Sciences. 2008; 8(1):115-20.

[95] Peixoto SB, Cladera-Olivera F, Daroit DJ, Brandelli A. Cellulase-producing Bacillus strains isolated from the intestine of Amazon basin fish. . Aquaculture Research 2011;42:887–91.

[96] Krogdahl Å, Hansen AKG, Kortner TM, Björkhem I, Krasnov A, Berge GM, et al. Choline and phosphatidylcholine, but not methionine, cysteine, taurine and taurocholate, eliminate excessive gut mucosal lipid accumulation in Atlantic salmon (Salmo salar L). Aquaculture. 2020;528:735552. 10.1016/j.aquaculture. 2020.735552.

[97] Kortner TM, Björkhem I, Krasnov A, Timmerhaus G, Krogdahl Å. Dietary cholesterol supplementation to a plant-based diet suppresses the complete pathway of cholesterol

synthesis and induces bile acid production in Atlantic salmon (Salmo salar L.). British journal of nutrition. 2014;111(12):2089-103. DOI: 10.1017/S0007114514000373.

[98] Twibell RG, Wilson RP. Preliminary evidence that cholesterol improves growth and feed intake of soybean meal-based diets in aquaria studies with juvenile channel catfish, Ictalurus punctatus. Aquaculture. 2004;236(1-4):539-46. 10.1016/j.aquaculture.2003.10.028.

[99] Chen Z, Zhao S, Liu Y, Yang P, Ai Q, Zhang W, et al. Dietary citric acid supplementation alleviates soybean meal-induced intestinal oxidative damage and micro-ecological imbalance in juvenile turbot, Scophthalmus maximus L. Aquaculture Research. 2018;49(12):3804-16. DOI: 10.1111/are.13847.

[100] Reyshari A, Mohammadiazarm H, Mohammadian T, Torfi Mozanzadeh M. Effects of sodium diformate on growth performance, gut microflora, digestive enzymes and innate immunological parameters of Asian sea bass (Lates calcarifer) juveniles. Aquaculture Nutrition. 2019;25(5):1135-44. DOI: 10.1111/anu.12929.

[101] Sarker SA, Satoh S, Kiron V. Supplementation of citric acid and amino acid-chelated trace element to develop environment-friendly feed for red sea bream, Pagrus major. Aquaculture. 2005;248(1-4):3-11. DOI: 10.1016/j.aquaculture.2005.04.012.

[102] Liu Y, Chen Z, Dai J, Yang P, Xu W, Ai Q, et al. Sodium butyrate supplementation in high-soybean meal diets for turbot (Scophthalmus maximus L.): effects on inflammatory status, mucosal barriers and microbiota in the intestine. Fish & shellfish immunology. 2019;88:65-75. DOI: 10.1016/j.fsi.2019.02.064.

[103] Krome C, Schuele F, Jauncey K, Focken U. Influence of a sodium formate/formic acid mixture on growth of juvenile common carp (Cyprinus carpio) fed different fishmeal replacement levels of detoxified Jatropha curcas kernel meal in practical, mixed diets. Journal of Applied Aquaculture. 2018;30(2):137-56. DOI: 10.1080/10454438.2017.1412845.

[104] Gao Y, Storebakken T, Shearer KD, Penn M, Øverland M. Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture. 2011;311(1-4):233-40. DOI: 10.1016/j.aquaculture.2010.11.048.

[105] Kortner TM, Penn MH, Björkhem I, Måsøval K, Krogdahl Å. Bile components and lecithin supplemented to plant based diets do not diminish diet related intestinal inflammation in Atlantic salmon. BMC veterinary research. 2016;12(1):1-12. DOI: 10.1186/s12917-016-0819-0.

[106] Mukhopadhyay Na, Ray A. Effect of fermentation on the nutritive value of sesame seed meal in the diets for rohu, Labeo rohita (Hamilton), fingerlings. 1999. DOI: 10.1046/j.1365-2095.1999.00101.x.

[107] Lazzarotto V, Corraze G, Leprevost A, Quillet E, Dupont-Nivet M, Médale F. Three-year breeding cycle of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet, totally free of marine resources: consequences for reproduction, fatty acid composition and progeny survival. PloS one. 2015;10(2):e0117609. DOI: 10.1371/journal.pone.0117609.

[108] Izquierdo M, Turkmen S, Montero D, Zamorano M, Afonso J, Karalazos V, et al. Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. Aquaculture. 2015;449:18-26. DOI: 10.1016/j.aquaculture.2015.03.032.

[109] Clarkson M, Migaud H, Metochis C, Vera LM, Leeming D, Tocher DR, et al. Early nutritional intervention can improve utilisation of vegetable-based diets in diploid and triploid Atlantic salmon (Salmo salar L.). British Journal of Nutrition. 2017; 118(1):17-29. DOI: 10.1017/S0007114517001842.

[110] Overturf K, Barrows FT, Hardy RW. Effect and interaction of rainbow trout strain (Oncorhynchus mykiss) and diet type on growth and nutrient retention. Aquaculture Research. 2013;44(4):604-11. DOI: 10.1111/j.1365-2109.2011.03065.x.

[111] Abernathy J, Overturf K. Expression of antisense long noncoding RNAs as potential regulators in rainbow trout with different tolerance to plant-based diets. Animal biotechnology. 2019;30(1):87-94. DOI: 10.1080/10495398.2017.1401546.