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Approaches to Use Anti-nutritional Factors Containing Plant Based Protein-rich Aquafeeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Replacement of the fish meal as a protein source in the aquafeeds is required these days due to the high cost of fish meal and its uncertain availability. Aquatic animals rely on fish meal for protein source so the protein source used in place of fish meal must not alter the nutritional quality of the feed. Therefore, such alternatives include animal and plant by- products such as meat, bone meal, rapeseed meal, soybean meal, corn gluten meal, a meal of protein- rich leaves, etc. However, the presence of anti-nutritional substances such as phytic acid, tannin, saponin, cyanide, oxalate, phytoestrogens, and gossypol limits the use of plant ingredients because ANFs have various side effects such as poor intestinal inflammation, oxidative stress, and decreased growth rate in many fish. To reduce the harmful effects several techniques such as solvent extraction, dry and wet heating, enzyme treatment, etc are used to reduce or eliminate such toxic components from Aquafeed. Amelioration strategies help in removal of anti-nutritional factors from plant based extract and are helpful in production of nutrient- rich and cost effective feed for fish for better growth and viability.

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1. INTRODUCTION

Aquafeeds are a variety of feeding materials used to feed aquatic animals with the optimum amount of essential nutrients. The various components of feed can be of animal or plant origin. High protein content along with other nutrients is major requirement for aquatic animals. At present, aquafeeds mostly rely on fish oil and fish meal sourced from wild forage fish. The diets of aquatic animals predominantly rely on fish meal for a protein source and fish oil for a lipid source rich in omega-3 fatty acids. Generally pelagic fish, also called as forage fish are used to derive fish meal and fish oil. However, the rising aquaculture demands are significantly increasing pressure on stocks of forage fish. The wild fisheries had reached its peak of production in 1995 following continuous decline. However, the global consumption of fish on a per capita basis is increasing per year at a rate of 1.5%. This has raised a serious concern by disturbing the food chains and food webs in the aquatic ecosystem. Moreover, the feed formulated by the use of fishes also has economic and ethical concerns. The uncertain availability and rising cost of fish meal as a source of protein in fish feed has become the main reason for its replacement with cheaper and readily available ingredients without reducing the nutritional quality of fish feed. Alternatives to fish meals include various animal byproducts like meat, poultry meal, bone meal, etc, and many plant ingredients rich in protein such as rapeseed meal, soybean meal, corn gluten meal, meal of protein-rich leaves, etc. However, plant protein sources also have significant limitations because such proteins have anti-nutritional substances. The anti-nutritional substances are part of the natural defense mechanism of plants in response to various pests and diseases. However, ANFs have a serious impact on the health of animals especially on lower vertebrates. The Major group of ANFs includes mimosine, protease inhibitors, phytic acid, tannin, saponin, cyanide, oxalate, phytoestrogens, gossypol, and non-starch polysaccharides [1]. ANFs are known to produce various side effects such as poor intestinal inflammation, oxidative stress, and decreased growth rate in many fish. The presence of other anti-nutritional factors (phytic acid, vicine, convicine) is also reported to cause poor palatability in fish [2]. Currently, several ways have been discovered to reduce or eliminate toxic components from Aquafeed. To remove the detrimental effects of antinutrients various processing techniques are used which include solvent extraction, dry and wet heating, enzyme treatment, etc [3]. However more research is required to study the physiological, nutritional, and ecological effects of ANFs by purification of individual antinutrients or mixtures of ANFs. Such studies will help know the optimum incorporation levels of plant-derived substances in Aquafeed and explore various treatment methods that reduce the negative effects of the ANFs. In this review article, we will discuss the amelioration strategies for the removal of anti-nutritional factors from plant-based extract to prepare aquafeed along with optimized nutrient contents. This in turn helps in the production of cost-effective and nutrient-rich feed for fish to improve growth and viability.

Antinutritional factors: The factors that interfere with the normal physiology and utilization of nutrients either directly or indirectly through products are called Anti-nutritional factors [4]. ANFs have a negative impact on the health of fish. The main source of Anti-nutrients in fish is the feed derived from plant sources, which are synthesized in plants for defense purposes. Based on mode of action, nutritional factors can be divided into four groups: (1) the ones that affect the digestion of protein. An example include- lectins, Protease inhibitors, Tannins, etc. (2) the ones that interfere with the utilization of minerals, For ex- gossypol, glucosinolates, phytates, oxalates, etc.(3) the ones that affect the utilization of antivitamins, For example- tannins. (4) Other ANFs include phytoestrogens, phorbol esters, mycotoxins, alkaloids, etc [5]. Various Antinutritional factors along with plant origin are presented in the Table 1.

Although nutritional value of all above mentioned plant sources is also very high. Lentils contains 20.6 – 31.4% proteins along with low fat and high fiber content, hence a good source of dietary protein. Pigeon pea has a rich amount of protein which can be used in regenerated meat products. Moringa leaves also contain various substances with anti-inflammatory and anti-oxidant properties along with high protein content i.e. 260g/kg. Rapeseed, which is an herbaceous plant cultivated for seed contains 17-26% of protein and and 40-50% of edible oil. It is also nowadays being used in aquaculture diet after pressing oil from it. Another versatile and

excellent protein-source legume is Faba bean. It is a low nitrogen- consuming legume whose seeds are being consumed worldwide being a good source of nutrients, fibers, and proteins. Byproducts of the brewing industry are also being used as potential sources of protein in aquaculture. BSG (Brewer's spent grain) is one of the main by-products of the brewing industry which is being used in Aquaculture feed due to its cost-effectiveness, high protein content, and easy availability.

Effect of Anti-nutritional factors on Fish Physiology: ANF can cause sublethal effects such as, poor feed conversion, lower feed intake, hormonal alterations, reduced growth rate, and organ damage. Tannins present in legumes and in high amounts in sorghum cause decreased protein digestibility as tannins precipitate protein thereby reducing the utilization of proteins in monogastric [12]. Diet with wet-processed faba bean isolates (BPI) inclusion (140g/kg) causes the lowered expression of gene sets of various physiological processes such as the immune system, digestive system, endocrine system,etc in Atlantic salmon [13]. Rapid changes in the gut transcriptome were also observed after switching the diet from fish meal to Soybean meal indicating the commencement of immune response followed by impairment of gut and intestinal barrier.

Protease inhibitors (ANF) inhibit the activity of many enzymes in the gastrointestinal tract by binding to chymotrypsin and/or trypsin and forming stable complexes thus resulting in decreased protein digestibility. Phytates also form meagrely decreasing the availability of protein for muscle formation. Consumption of plant-based feed containing tannins can cause a reduction in feed intake, nutrient utilization, and growth. Not only this microbiota of gut changes on giving plant-based fish feed example when rainbow trout (*Oncorhynchus mykiss*) was given diets having plant ingredients such as peas, soybean, canola, etc., it changes the gut microbiota which has a negative effect on the health of fish [14]. Replacement of fishmeal with plant protein-containing feed in Atlantic salmon (*Salmo salar*) leads to significant alteration of gut microbes composition and decreases α-diversit [15]. Spirochaetes and the families Moritellacea, Helicobacteraceae, Psychromonadaceae, and Bacteroidaceae were significantly decreased in the groups fed with 80% plant protein diets when compared to control. However, no significant morphological change was seen in the fish fed with a plant-based protein diet and did not show signs of inflammation or enteritis in the gut. But were found to have wider villi and wider intestinal walls compared to FM fish(ds). Common carp (*Cyprinus carpio* L.) fed with CON (wheat gluten protein-based control diet without lysine supplementation) diet Showed low intestinal folds and more abundant mucous cells compared to the control groups. Both the changes point toward disturbances in the structure of mucosa, intracellular digestion, and metabolic disorders. The number of intestinal mucous cells also increased in carp and salmon fed with a diet containing fat from soybeans. Similar results were seen at the time of malnutrition or starvation with an increase of the goblet cells in the intestine, followed by low intestinal folds and, a reduction in absorptive surface, which resulted in lower body mass. also intestinal epithelium of fish fed CON showed a higher rate of proliferation and apoptosis. Disturbed cellular homeostasis, increased proliferation, and apoptosis of intestinal epithelium were also observed in Atlantic salmon fed with a soybean meal diet. Phytoestrogens (Plant sterols) are also reported to act as endocrine disruptors and negatively affect the health of the fish [16].

Plant based Aquaculture feed is reported to affect multiple genes and signalling pathways. Studies on Atlantic salmon suggested that when fish were fed with single plant protein diet with fish meal or a mixed plant protein diet (Soybean protein concentrate or Faba beans protein concentrate) with fish meal phenotypic and physiological changes appeared which are as follows: Similar growth rate is observed was observed in the group fed with the same amount of fish meal (22%) but different SPC and BPC except the group fed with 45% BPC showed slow growth rate. High protein content is also recorded in fish feed with a mixed plant protein Diet as compared with a single protein diet. Histological examination suggested that fish fed with B45 and SBM caused mild and moderate enteritis respectively. When gut transcriptome response was also analysed It was found that single plant protein diet i.e. S45 and B45 diets caused change in 140 (17 pathways) and 254 genes (12 pathways) respectively. The 17 pathways affected by S45 diet include estrogen biosynthesis, melatonin degradation, acetone degradation, etc. A B45 diet affected pathways involved in the migration of granulocytes, endocytosis signalling pathway, tight junction signalling pathway, integrin- linked kinase signalling pathways etc. Intestinal uptake of

Table 1. Various Antinutritional factors along with plant origin

	Antinutritional factor	Plant source	Effect
1.	Cyanonegic Glycoside, Hemagglutinin, Alkaloid, Tannins	Pigeon pea [6]	Bio-availability of Proteins is inhibited
2.	Protease inhibitors, amylase inhibitors, lectins, convicine and vicine, etc.	Faba beans [7]	Poor palatability, oxidative stress, intestinal inflammation etc.
3.	Hydrocyanic acid, phytic acid, amygdalin, and tannins.	Plum kernels	Effects bioavailability of volatile and phenolic compounds
4.	Lignin and fibre	Brewer's spent grain	Reduce digestive performance
5.	Oxalates	Cassava [8]	affecting Ca and Mg bioavailability and forming complexes with proteins, which inhibit peptic digestion.
6.	Carbohydrates, glucosinolates, phytates, phenolic compounds etc	Rapeseed [9]	lower digestibility
7.	High non-hemolytic saponins,tannins, oxalates and phytates	Moringa Leaves [10]	Reduced growth and poor feed efficiency
8.	Phytic acid, lectins , tannins, and protease inhibitors	Lentil [11]	lower the protein digestibility

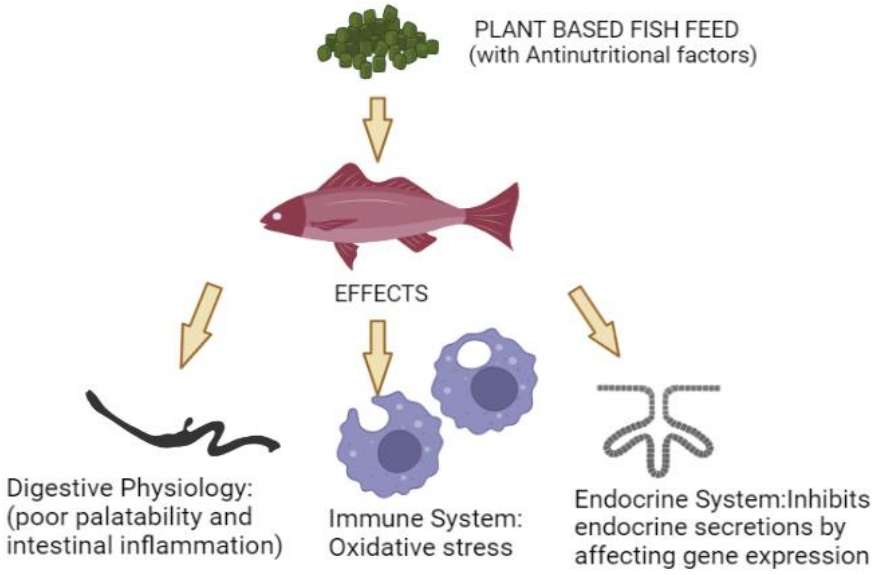


Fig 1. Overview of effect of Antinutritional factors on Fish physiology

foreign antigens, allergens, or other harmful macromolecule increased cellular permeability due to the formation of holes as a result of saponin binding to membrane cholesterol is also reported in some studies [5]. Therefore, antinutritional factors should be removed before using the protein-rich plant material in fish feed.

2. AMELIORATION STRATEGIES

Aquaculture is growing tremendously, and demand for aquafeed is increasing with time. Plants' abstracted feed has a high protein and can fulfil growing needs. Hover, plants have many antinutritional factors that are harmful to fish and cause many side effects like interfering with food utilization, growth, health, and reproduction of animals. Several physical and chemical methods along with various biotechnological techniques can be used for reducing or removing the effect of the antinutritional compounds. Physical methods involve processes such as dehulling, washing, soaking, heating etc where whaereas chemical methods involve the use of various solvents, acids, enzyme treatment etc. The suitable treatment to be used for mitigation also depends upon the type of anti-nutritional factor. Understanding the functioning of anti-nutritional factors can help in neutralizing the side effects and can solve the problem:

Protease Inhibitors: Bind with many digestive enzymes like chymotrypsin or trypsin, making them partially or fully inactive but the effect can be minimized as trypsin can be destroyed by roasting as all the protease inhibitors are heat labile.

Lectins or Haemagglutinin: Lectins or haemagglutinin cause a reduction in nutrition absorption from the gut or alimentary canal. Internal haemorrhages are sometimes caused by such compounds and can also cause reduction in growth. Lectins are also heat labile and can be destroyed by heat processing and roasting.

Tannin reduces: Tannin reduces the absorption of vitamin B12. Hydrolyzed tannins can cause toxicity to the organs like the liver and kidney. Tannin could be removed by a simple process of dehulling the seeds to remove the outer layer which is tannin-rich, by treatment seeds with alkali and autoclaving can also reduce the tannin level.

Phytate's: Phytate's presence in feed decreases phosphorous availability. Phytic acid can also affect the bioavailability of zinc and many other divalent cations so it becomes necessary to fortify the feeds with supplemental zinc when phytic acid ingredients are present. Deficiency of zinc leads to bilateral cataracts, damage of the intestine, reduction in absorption of nutrients, reduced feed efficiency, growth, and affects the proper functioning of the thyroid. Milling and removal of the outer layer can reduce the phytate contents. Fermentation can also reduce the

phytic acid content of some grains due to the action of phytases produced by lactic acid bacteria or yeast [17]. Incorporation of more minerals like zinc or supplementation with the enzyme ‘phytase’ in phytate-rich ingredients feed can neutralize the negative effects [18].

Gossypol: Gossypol forms a gossypol protein complex and may cause a deficiency of a few amino acids like ‘methionine’, which has a significant role in fat metabolism. Gossypol diminishes the bioavailability of a few other limiting amino acids like ‘lysine’. Different fish species have variable sensitivity to gossypol. Many studies report that gossypol reduces the activity of succinic dehydrogenase enzyme and cytochrome activity. Gossypol reacts with iron and leads to the formation of an inactive ferrous gossypol complex. So dietary iron can be successfully used to counter or neutralize the side effects of gossypol in formulated fish feeds for gossypol-sensitive fish species.

Oxalates: Oxalates It make some of the minerals unavailable to fish. Enrichment or suitable fortification with Ca salt can be a method to overcome the problem.

Glucosinolate: Glucosinolate compounds are not very harmful by themselves but by hydrolysis from enzyme 'myrisinase’ in poorly processed oilcake or produced by intestinal microflora, a variety of products can be formed such as the thiocyanate ion, isothiocyanate ion, goitrins, nitriles etc. Improved genetic varieties of rapeseeds developed which contain low levels of glucosinolate. The remaining levels of glucosinolate can be removed by solvent extraction of the oil from oilseed and heat treatment by pressure cooking over a long time.

Saponins: Saponins are highly toxic to fish when present in water or are spread on the water's surface. The detergent action of saponins causes damage to the respiratory epithelium of fish gills. Sometimes saponin forms complex with other antinutrients, which leads to the inactivation of various toxic effects of both substances. For example, tannin and saponin simultaneously form a complex and may result in the loss of the individual toxicity of both substances [19]. As saponins are highly soluble in water, keeping them dipped in water for some time can remove most of the saponins.

The knowledge of the important biological effects of antinutritional factors on farmed fish now seems essential. Developing methods for inactivating or destroying, reducing the impact of anti-nutritional factors, or overcoming its effects will become increasingly important in the expansion of aquafeed formulation and production.

3. STRATEGIES USED TO REDUCE FOOD LEVELS OF ANTINUTRIENTS

Different traditional and technological processing ways such as soaking, debranning, milling, roasting, cooking, fermentation,and germination can be used for the reduction of anti-nutritional components in the feed.

Milling: Milling is the most commonly used traditional way to separate the outer layer from the grains. The milling process can remove many anti-nutrients like phytic acid, lectins, tannin etc., which are present in the bran part of grains, but this technique has one main disadvantage that it also removes important minerals along with antinutritional compounds [20]. Many studies on millet milling conclude that pearl millet's chemical composition changes.

Soaking: Soaking is one of the important methods for removing the antinutrient compounds from food because soaking has another advantage as it reduces cooking time. Soaking also increases the release of enzymes like endogenous phytases, which are present in plant products. Soaking generally makes available moist conditions in nuts, grains which is essential for germination in turn it results in associated reductions in many enzyme inhibitors levels and reduces other anti-nutrients,and enhances the nutritional value, digestibility of the food. Many of the anti-nutrients have a water-soluble nature, so the removal of ANFs can be enhanced from foods through the process of leaching. Soaking commonly enhances the level of hydrogen in legumes and cereals, which softens them and also increases the activity of endogenous enzymes like phytase which can be further enhanced by processing methods like cooking or heating [21]. Studies have shown that soaking of 5 hours reduced 27.9% and soaking of 20 hours reduced 36.0% of phytic acid at room temperature in Mucana flagellipes. Soaking increases the activity of phytase which results in the reduction of phytate content in the grains. One of the disadvantages of soaking and fermentation is this process reduces phytochemicals by leaching water-soluble vitamins and minerals in grains and legumes [22].

Autoclave and cooking Autoclave: Autoclave and cooking Autoclave is generally used for various heat treatments. When this process is used on cereals and other plant-based foods, it can activate enzymes like phytase and also increase the acidity of food [23]. Many of the foods show health benefits when autoclaved before consumption. Rehman and Shah, 2005 studies concluded that boiling grains can reduce anti-nutrient compounds and enhance nutritional value. Savage and Mårtensson, 2010 also stated that the oxalate level of taro leaves reduced by up to 47% when boiled for 40 minutes in water, even though no significant reduction was observed in oxalate content due to baking for the same time at 180 °C. Roasting is also considered a good method to decrease the trypsin inhibitors in the food.

Fermentation: Fermentation can be used for the reduction of bacterial contamination of foods. Fermented millet products are usually recommended as probiotics to treat diarrhoea Fermentation is a metabolic process which involves the oxidation of sugars to produce energy; fermentation also increases the absorption of minerals from plant-based food. The phytic acid present in kernels forms a complex with various metal cations like iron, zinc, calcium and proteins. The complexes are generally degraded by enzymes, which work at an optimum pH maintained during the fermentation process. So this kind of process decreases the phytic acid from food and releases the soluble iron, zinc and calcium, hence enhancing the nutritional level of food [24]. In some of the studies fermentation of cereals with the help of lactic acid bacteria (LAB) has been used to increase free amino acids. And it was concluded by Mohapatra et al. 2019 that fermentation can be used to improve the nutritional value of grains because it enhances the availability of essential amino acids such as lysine, methionine and tryptophan.

Germination: Germination is considered a suitable method for decreasing the anti-nutrient content of plant-based foods. Germination of seeds mostly activates the phytase enzyme, which destroys phytate and decreases phytic acid concentration in the food samples. Germination changes various nutritional levels, physical features and biochemical property of the feeds. For the reduction of cereals' anti-nutritional content and hence this method is most commonly used for the reduction of antinutritional factors from a feed. One of the studies stated that after malting of millet samples for 72 h and 96 h, it was found that the phytic acid component was decreased to 23.95 and 45.3%, respectively In work done by Azeke et al., 2011 it was concluded that the phytate level of cereal grains decreased significantly after 10 days of germination.

Therefore, the quality of plant-based feeds can be improved by the utilization of various processing methods, especially germination and fermentation.

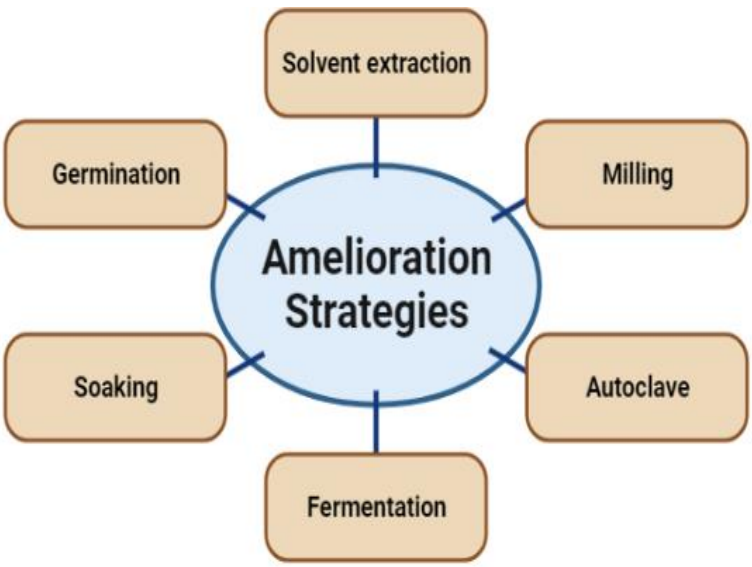
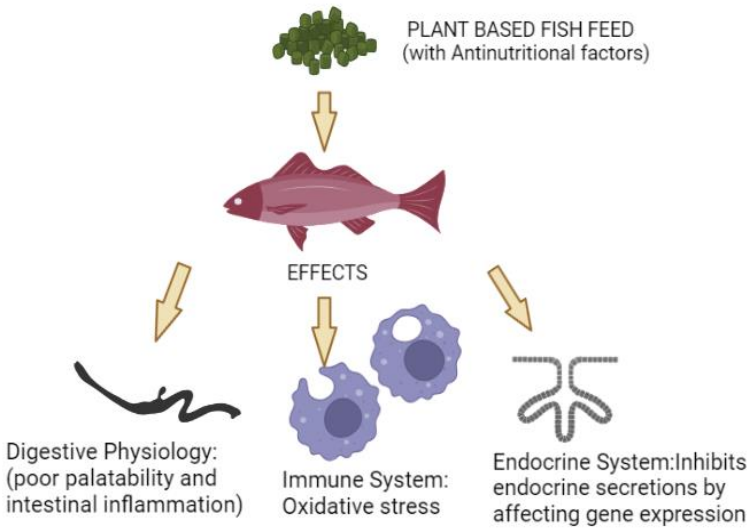


Fig 2. Amelioration strategies to reduce the amount of Antinutritional substances

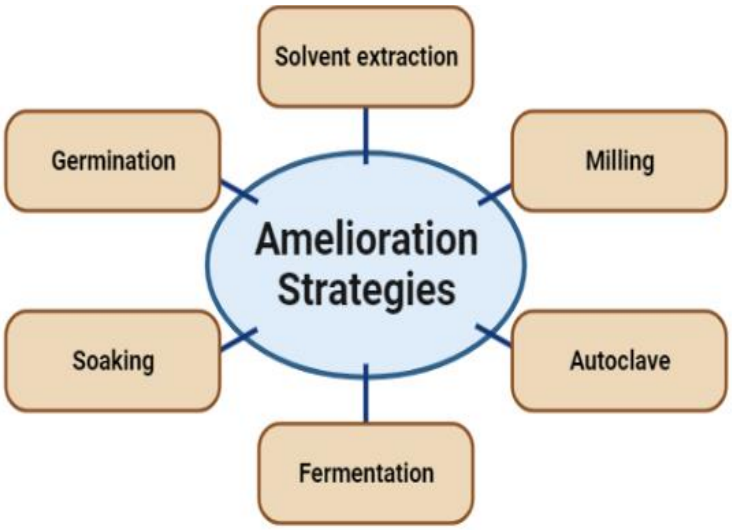
Table 2. Antinutritional factor with plant source and their effect

	Antinutritional factor	Plant source	Effect
1.	Cyanonegic Glycoside, Hemagglutinin, Alkaloid, Tannins	Pigeon pea [6]	Bio-availability of Proteins is inhibited
2.	Protease inhibitors, amylase inhibitors, lectins, convicine and vicine, etc.	Faba beans [7]	Poor palatability, oxidative stress, intestinal inflammation etc.
3.	Hydrocyanic acid, phytic acid, amygdalin and tannins.	Plum kernels	Effects bioavailability of volatile and phenolic compounds
4.	Lignin and fibre	Brewer's spent grain	Reduce digestive performance
5.	Oxalates	Cassava [8]	affecting Ca and Mg bioavailability and form complexes with proteins, which inhibit peptic digestion.
6.	Carbohydrates, glucosinolates, phytates, phenolic compounds etc	Rapeseed [9]	lower digestibility
7.	High non-hemolytic saponins,tannins, oxalates and phytates	Moringa Leaves [10]	Reduced growth and poor feed efficiency
8.	Phytic acid, lectins , tannins and protease inhibitors	Lentil [11]	lower the protein digestibility



A.

Fig. 3. A. Overview of effect of Antinutritional factors on Fish physiology



B.

Fig. 3. B. Amelioration strategies to reduce the amount of Antinutritional substances

4. FUTURE PERSEPECTIVES

The anti-nutritional factors may have adverse effects in animals like fishes as ll as human health. The varsity of adverse effects generally depends on the concentration of the antinutritional molecule. The antinutritional factors may be the important component of plant metabolism and expression of ANFs also varies from one crop to the other, therefore, complete elimination of the antinutritional factors may not be the solution. Breeding programmes involved in eliminating antinutritional are also compromising in terms of yield. Therefore, advance molecular such as techniques genomics-assisted breeding, gene editing etc emphasized on individual crop-based approaches may help in to reducing the concentration of the antinutritional factors and produce foods with minimum constraints in future [25].

5. CONCLUSION

Considering the rising fisheries sector and thereby increased demand for cost-effective and nutrient-rich aquafeed, protein-rich plant ingredients appear to be promising alternatives to fish meal. Apart from the various ANFs found in plants which negatively impact the health of

fishes, plant ingredients are of utmost importance as Lentils contain 20.6 – 31.4% proteins along with low fat and high fiber content and Rapeseed contains 17-26% of protein and 40-50% of edible oil. Other legumes such as Faba beans and byproducts of the brewing industry are also excellent sources of protein. Moringa leaves also contain various substances with anti-inflammatory and anti-oxidant properties along with high protein content . To remove the ANFs various techniques are being used such as solvent extraction, soaking, dehulling, dry and t heating, enzyme treatment, etc. Hover, a better understanding of the functioning of the antinutritional factors present in plants can help in neutralizing the side effects of ANFs in plant-based diet of fish. Purification of individual antinutrients or mixtures to understand the physiological effects of ANFs requires more research as this kind of study will help determine the optimum incorporation levels of plant-derived substances in Aquafeed thereby producing cost-effective feed which is the major operational input of the aquaculture industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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