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Anti-Nutritional Factors in Plant-Based Aquafeed Ingredients: Effects on Fish and Amelioration Strategies

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ABSTRACT

Limited supply and high demand of fish meal made it imperative to use plant derived feed ingredients such as seeds of legumes (lupin and peas), oil seed cakes (soy bean, cottonseed, and rape seed), cereals (corn, rice and wheat), meal of protein rich leaves, concentrates and isolate of non-edible oil seeds (jatropha, castor, karanj and neem) as a fish feed ingredients. However, the major challenge in utilising the protein rich plant derived ingredients as fish feed is the presence of anti-nutritional factors. The most widely distributed anti-nutritional factors among potential alternatives are protease inhibitors, phytic acid, saponin, tannin, cyanide, oxalate, gossypol, non-starch polysaccharides, phytoestrogens, mimosine. We need to remove or ameliorate the effects of these anti-nutritional factors for the incorporation of the plant ingredients. There exists a species-specific tolerance limit to each anti-nutrients which needs to consider before determining their amelioration techniques. The effects of these anti-nutrients along with the techniques employed to remove them have been discussed in this article.

KEY WORDS: ANTI-NUTRITIONAL FACTORS; AMELIORATION STRATEGIES; FISH; PLANT INGREDIENTS

INTRODUCTION

Aquaculture has become an important sector for improving food security, raising nutritional standards and alleviating poverty, especially in the light of increasing global population which is projected to reach 10 billion by 2050 (FAO, 2016). Global aquaculture sector grows faster than any other food producing sector with a growth rate of 5.8% during 2001-2016 (FAO, 2018). To sustain such high rates of increase in aquaculture production, a matching increase in the levels of production of fish feeds is required.The most immediate challenge to aquafeed industry is the availability of quality feed ingredients that not only meet the nutritional requirements of fish but also minimise production cost, limit environmental impacts and enhance products quality. In this regard, fish meal is the most utilised traditional dietary protein ingredient in aquaculture diets because of its high protein content, balanced amino acid profile, high digestibility and palatability (Drew et al., 2007). However, inconsistent supply, localised production, depleted fish stocks, greater demand, and rising prices have made it imperative for fish feed industry to look for more economical and sustainable alternatives.

In search of ingredients to replace totally of partially the fish meal, soybean meal is the most commonly used alternative in many aquaculture species (Gopan et al., 2019b). The nutrient quality such as amino acid profile and high digestibility are the reasons for choosing soyabean as the alternative for fish meal especially in

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freshwater species. However, using soyabean as a fish meal replacer is also not sustainable as it has high demand and competition from other sectors holds high cost and its incorporation in fish feed will erode the expected profit. Similarly, most commonly used other plant products such as ground nut, mustard, rice bran and other legumes seeds possess same problem. Thus, finding alternatives to commonly used plant derived ingredient is a major concern for nutritionists. Another option is to utliise the by-product of commonly used plant ingredients. The plant-derived nutrient sources and their by product may become very good alternatives but they are known to contains a wide variety of anti-nutritional substances. The presence of this naturally occurring anti-nutritional substance are the sole factor which limit their utilisation in the fish feed (Gopan et al., 2019a, b). These anti-nutritional factors are the part of defense system of plants against different diseases and pests. However, they pose several adverse effects on animals, especially on the non-ruminants.

Table 1. Important anti-nutritional factors present in plant derived nutrient source

Plant-derived nutrient source	Anti-nutritional factors
Ground nut oil cake	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, NSP
Mustard oil cake	Glucosinolates, tannins
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitor
Linseed oil cake	Cyanogens, Phytic acid, phytoestrogen, anti-vitamins (anti-thiamin& anti-pyridoxine)
Rubber seed oil cake	Cyanide, Phytic acid, Tannin, Trypsin inhibitor
Neem seed oil cake	Azadirachtin, Cyanide, Phytic acid, oxalate, Tannin
Karanja oil cake	Karanjin, phytic acid, Tannin, Trypsin inhibitor
Caster seed oil cake	alkaloid ricinine, tannin, phytate and oxalate
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, allergens
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, anti-vitamins, cyclopropenoic acid
Sesame meal	Phytic acid, protease inhibitors
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, anti-vitamins
Leucaena leaf meal	Mimosine
Alfalfa leaf meal	Protease inhibitors, saponins, phytoestrogens, anti-vitamins
Jatropha curcas meal	Phorbol esters, Trypsin Inhibitors, Lectin, Cyanogenic glycosides, glucosinolates, amylase inhibitors, Saponins, Tannins, Phytates, Non-starch polysachharides
Sweet potato leaf meal	Phytic acid, tannin, oxalate, saponin, trypsin inhibitor, alkaloid, cyanide
Hygrophila spinosa leaf meal	Phytic acid, oxalate, tannin, alkaloids
Sesbania aculeata leaf meal	Phytic acid, tannin, alkaloids, oxalate Rice Protease inhibitors, lectins, phytic acid, anti-vitamins
Wheat	Protease inhibitors, lectins, phytic acid, NSP
Corn	Protease inhibitors, lectins, NSP
Duckweed	Protease inhibitors, Cyanogens, tannin, gossypol
Grass pea	Protease inhibitors, phytic acid, tannin, lathyrogen
Azola	Protease inhibitors, phytic acid, tannin, gossypol
Pistia	Protease inhibitors, saponins, tannin
Eichornia	Protease inhibitors, phytic acid, cyanogen, saponins, tannin
Chick pea	Protease inhibitors, phytic acid, cyanogen

Anti-nutritional factors: The anti-nutritional factors (ANFs) may be defined as substances which by themselves or through their metabolic products arising in living systems, interfere with nutrient utilisation and affect the health and production of animals (Makkar, 1993). The substances which produce direct health effects are called toxic principles. In nature, plants can synthesise certain antimetabolites which exert a deleterious effect upon ingestion by animals. The plant synthesises these chemical substances for self-defence.

Plants contain a wide range of compounds which can have beneficial or harmful effects on organisms consuming them by influencing the metabolic pathways. ANF causes sublethal effects such as lower feed intake, poor feed conversion, reduced growth rate, hormonal alterations, and organ damage (Francis et al., 2001). Thus, the anti-nutritional factors restrict the use of the plant ingredients in the feed. Although plant-based ingredients are deficient in some essential amino acids, it can be rectified by supplementing them in the diet. However, the ANF should be eliminated from the ingredients to use different plant-derived materials as alternative feed ingredients.

Classification of Anti-nutritional Factors of Plant ingredients

According to the chemical nature, ANFs in plant ingredients can be classified into four major groups such as,

1. Proteins: includes protease inhibitors, hemagglutinins, toxic amino acids and food allergens
2. Glycosides: which includes goitrogens, cyanogens, saponins and estrogens
3. Phenols: Gossypol and tannin
4. Other phytochemicals, such as phytic acids, anti-vitamins, and anti enzymes.

According to the mode of action ANFs are broadly divided into four groups such as 1) which affect protein utilisation and digestion example protease inhibitors (trypsin inhibitor, chymotrypsin inhibitor), tannins, and lectins (haemagglutinin) 2) Which affects mineral utilisation such as phytates, gossypol, oxalates, glucosinolates 3) Which affects utilisation of anti-vitamins such as tannins 4) miscellaneous substances like mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitising agents, phytoestrogens and saponins and phorbol esters(Francis et al., 2001)

Based on the ability to withstand the thermal processing, which is commonly employed to destroy them the ANFs are classified into heat-labile factors represented by protease inhibitor, phytates, lectins, goitrogens and anti-vitamins whereas heat-stable factors such as saponin, non-starch polysaccharides, antigenic protein, estrogens and phenolics compounds. ANFs may also be classified as per the source such as endogenous, which includes ANFs which is present in the feed ingredient itself. Such as protease inhibitor, tannin, lectin, phytate, gossypol, oxalate. Exogenous ANFs which includes substance produced by extraneous agents represented by mycotoxins produced by the fungus. Aflatoxins, zearalenone, deoxynivalenol, fumonisins, ochratoxin and trichothecene, are examples for mycotoxins. Aflatoxins produced by Aspergillus flavus is the most common and dangerous one in fish feed. Important ANFs present in alternative fish feed ingredients are depicted in the table 1.

Table 2. Important anti-nutritional factors and their harmful effects, dietary tolerable level and amelioration techniques.

Anti-nutritional factors	Harmful effects	Dietary tolerable level	Amelioration techniques
Protease Inhibitors	i)Pancreatic hypertrophy /hyperplasia ii) Reduced protein digestion and amino acid utilization iii) Reduced growth	i) Salmonids: <5 mg/g ii) Nile tilapia: < 1.6 mg/g iii) Carp: > 8.3 mg TI/g iv) Channel catfish: 2.2 mg/g	i) Moist heat treatment (autoclaving) for 15–30 min ii) Extrusion or steam cooking iii) Fermentation iv) Germination v) Supplementation of essential amino acids, especially S-containing amino acids, to compensate the unavailable amino acids.
Phytic acid	i) Reduced protein, carbohydrate and minerals utilisation and growth in ii)reduced muscle ash content iii)skeletal deformity iv) reduced thyroid function v)Promotion of cataract formation vi) Abnormal pyloric caecal structure resulting in depressed absorption of nutrients vii) Increased mortality.	i)Carps, tilapia, trout, fish salmon and shrimp: <5 g/kg or <0.5%	i) Dietary phytase supplementation ii) Fermentation of feed ingredients yeast or lactic acid bacteria iii) Milling of outer layer iv) Supplementation of mineral premix v) Microwave irradiation & E-beam irradiation vi) The additional supplementation of Zn to prevent cataract vii) Aqueous extraction (18h) viii) Moist heating (120oC for 2h)

Table 2 Continue

Glucosinolates	i) Progoitrin and epi-progoitrin impair palatability & reduced feed intake ii) The thiocyanates, allyl isothiocyanate and Goitrin interfere with iodine availability being the most potent antithyroid agents iii) Antithyroid activity leads to less production of T3 & T4 that affects metabolism resulting in depressed growth iv) Nitriles are known to affect liver and kidney functions with severe damage	i) Common carp (Cyprinus carpio) and other fish: <0.4 mg/g diet or 3.6 μmol/g diet ii) The carnivorous fish species are generally more susceptible to glucosinolate toxicity than omnivorous/herbivorous fish species	i) Water Extraction is a cost-effective method however leaching of essential nutrients is a problem ii) Heat treatment (extrusion cooking & wet pressure -cooking) iii) Readily removed by extraction with dilute alkali or organic solvent mixtures iv) Treatment with Copper sulphate solution v) Microwave irradiation & E-beam irradiation vi) Iodine supplementation in the diet of and ruminants vii) Selective breeding programmes to yield low glucosinolate rapeseed/ mustard varieties.
Saponins	i)Reduce palatability and feed intake ii)reduced feed efficiency & growth iii)reduced reproductive performances iv) respiratory distress v) death.	i) Carp and other fish: < 1 g/kg of diet ii) iii) Ornamental fish: yet to be established.	i) Aqueous extraction but leaching of nutrients should be taken care of ii) Extraction with ethanol iii) Cholesterol supplementation.
Tannins	i) Due to undesirable bitter taste they reduce palatability and feed intake ii) Reduced feed efficiency & growth iii) Damage of liver and kidney lead to death.	i) Ruminants: up to 15000 mg/kg feed (1.5%) ii) Laying hens: up to 10000 mg/kg feed (1%) iii) Rabbit: up to 10000 mg/kg feed (1%) iv) Pig: up to 1500 mg/kg feed (0.15%) v)Up to 15 mg/kg feed is safe for all animal species	i) Dehulling of seeds to remove the tannin-rich outer layer ii) Treatment with alkali iii) Treatment with ferrous sulphate (oxidising agent) iv) Treatment with tannin complexing agents polyethene glycol v) Soaking and drying and heat treatment (autoclaving) vi) Fermentation with lactic acid bacteria vii) Microwave radiation and E-beam irradiation.
Cyanogens	i) reduced feed efficiency and growth ii) death in excess dose	i) Livestock: up to 100 mg/kg feed on DM basis (0.01%). The lethal dose of HCN for cattle and sheep is 2.0-4.0 mg per kg body weight ii) Human: The lethal dose of HCN taken by mouth in humans has been estimated to be between 0.5 and 3.5 mg/kg body weight	i)water soaking(24h) followed by sun-drying ii) Boiling followed by sun-drying iii) Roasting iv)Microbial fermentation v) Grinding followed by sun-drying vi)Microwave radiation and E-beam irradiation.

Table 2 Continue

Gossypol	i) Constipation ii) Depressed appetite iii) Loss of weight iv) The toxic, systemic effects of gossypol exhibited through reduction of haematocrit, haemoglobin, reproductive capacity as well as lesions in the liver, kidney, spleen and gonads may develop v) Death.	i) Fish: <20 mg/kg feed recommended for all animals but some fish can tolerate the level between up to 100-<300 mg/kg diet	i) Heat treatment: Roasting, extrusion ii) Irradiation: Gamma or E-beam irradiation iii) Fungal fermentation iv)Nutritional supplementation: a) Deficient amino acids, such as cysteine, lysine, and methionine b) Ferric sulphate c) Sodium selenite d) Vitamin E
Haemagglutinins or lectins	i) agglutination of RBC ii)reduction in the absorption of nutrients from the gut or alimentary canal iii)Internal haemorrhages iv) reduction in growth.		Heat treatment. Moist heat treatment (autoclaving) is more effective than dry heat treatment.
Oxalate or oxalic acid	i) formation of calcium magnesium oxalate stones in the kidney concomitant kidney failure ii) growth and immunity depression	ruminants < 2% dietary oxalates and for non-ruminants < 0.5% to avoid oxalate poisoning	Heat treatment
Phorbol ester	i) Feed rejection ii) growth reduction, severe damage of intestine liver and kidney, death at high dose	Common carp above 3.75 ppm caused an adverse effect	Autoclaving, Fermentation,

Major ANFs and their effects and amelioration: Based on the chemical nature of the ANFs and amount present in the ingredients, their efficacy against nutrient utilisation and fish health. Possible mode of action for different anti-nutrients are discussed below based on the available information. Harmful effects of ANFs dietary tolerable level and their amelioration process are discussed in table 2.

Protease Inhibitors: The factors which cause an adverse effect on the nutritional value of proteins are known as protease inhibitors. These are the entities that exhibit the ability to inhibit the proteolytic activity of some enzymes. They are found in the entire plant kingdom, particularly among the legumes, such as soybean (Norton, 1991). Biochemically, they fall under two categories such as i) Kunitz inhibitors with a molecular weight of 20,000 to 25,000 Da with few disulfide bonds which inhibit trypsin and ii) Bowman-Birk inhibitors with a molecular weight of only 6000 to 10,000 Da, rich in cysteine and inhibit chymotrypsin as well as trypsin at the independent binding site (Makkar et al., 2007). Commercial soybean products mostly show trypsin inhibitors (TI) in the range of 2–6 mg/g, averaging 4 mg/g. Protease inhibitors inhibit the activity of enzymes within the gastrointestinal tract of animals by binding with chymotrypsin and/or trypsin to form stable complexes thus preventing access to the active site of the enzyme leading to decreased protein digestibility.

Phytic acid or Phytate: Phytates or Phytic acid is one among the most potent anti-nutritional factors in plant feedstuffs. It is a common storage form of phosphorus in plant seeds. Phytate (hexaphosphates of myo-inositol) can chelate with di- and trivalent mineral ions such as phosphorus, calcium and magnesium, trace elements such as iron and zinc, and protein and amino acids (D'Mello et al., 1991). Commonly used and potentially usable plant-derived fish feed ingredients such as soybean meal, rapeseed meal, and sesame meal contain 10–15, 50–75 and 24 g/kg phytate, respectively (Francis et al., 2001). Inclusion of phytate containing ingredients in the diet has negatively affected the growth in commonly cultured fish species as most of the fishes do not have endogenous enzymes to break down phytate and release nutrients. Thus, they pass through the gut undigested. Therefore, more significant proportions of valuable nutrients from plant sources are becoming unavailable for aquatic animals and are wasted as excreta. Phytates also form meagrely digestible phytate–protein complexes, resulting in reduced digestibility and availability of protein for muscle growth (Richardson et al., 1985; Makkar et al., 2007). Growth and nutrient utilisation efficiency of different fish species fed phytate-containing diets were reported to be adversely affected (Francis et al., 2001).

Saponins: Saponins are triterpenoid or steroidal glycosides which are characterised by their hemolytic and foam producing properties and impart a bitter taste. They are found in feed ingredients like oilseed cakes, legumes etc. Mostly, the leguminous seeds contain 18-41 mg/kg, and defatted soya-bean meal contains 67 mg/kg of saponin (Fenwick et al., 1991). Even though saponins are found in many of the potential alternative fish feed ingredients, they are highly toxic to fishes due to their interaction with cellular membrane component. It can hemolyse red blood cell with nonspecific interactions with membrane proteins. Saponin is the active ingredient in the mahua oil cake which is used as a fish poison to kill unwanted fishes in the culture system.

Tannins: Tannins are secondary compounds of various chemical structures widely occurring in the plant kingdom and are divided into hydrolysable and condensed tannins. They interfere with the digestive processes either by binding the enzymes or by binding to feed components like proteins or minerals and reduce the absorption of vitamin B12 (Francis et al., 2001). Plant ingredients tested as alternative nutrient sources in fish feed such as rapeseed meal, pea seed meal, mustard oil cake, do contain tannin. Hydrolysable tannin can be broken down by acid, alkali, and some hydrolytic enzymes present in the biological systems, thereby forming smaller compounds that can enter the bloodstream (Francis et al., 2001). Feeding on diets containing high levels of hydrolysable tannins causes toxicity to vital organs and lead to the death of the animal (Francis et al., 2001; Makkar et al., 2007). Contrarily, condensed tannins are highly resistant to degradation. Consumption of plant ingredients containing condensed tannins can cause a reduction in feed intake nutrient utilisation and growth (Makkar et al., 2007).

Oxalates: Oxalate is an anti-nutritional factor which affects mineral utilisation; it is present in appreciable amounts in fish feed ingredients (Francis et al., 2001). Oxalic acid is a metabolic product formed through several pathways in plants and animals. .Oxalates of monovalent ions, such as sodium, potassium or ammonium are well soluble in water however those oxalates formed with divalent ions, such as calcium, magnesium and iron are almost insoluble (Libert and Franceschi, 1987; Savage et al., 2000).Oxalates chelates with dietary calcium and with other divalent metals with specific concentration (Abara et al., 2000). Thus calcium is limited, unavailable for absorption, making it less bioavailable for bone formation and various metabolic activities as well as for the cofactor requirements of many enzymes (Hajra et al., 2013). In mammals, long-term exposure to a high-oxalate diet may lead to the formation of calcium magnesium oxalate stones in the kidney, which can cause urine flow problems or kidney failure (Noonan and Savage, 1999). According to Rahman et al. (2013), oxalate consumption should be less than 2% for ruminants dietary to avoid oxalate poisoning and less than 0.5% for non-ruminants.

Cyanides: Hydrogen cyanide also called prussic acid, is an organic compound with the chemical formula HCN. It is a colourless, extremely poisonous liquid that boils slightly above room temperature, at 25.6°C. Cyanides are one of the most common anti-nutritional factors in plant kingdom which exist in the form of cyanogenic glycosides (or cyanogen) (Makkar et al., 2007). Cyanogens are found in high concentrations in several pulses, root crops, such as cassava, and some oil seeds, such as linseed, which have been tried as fish feed ingredients (Francis et al., 2001). Cyanogens are glycosides of sugar and cyanide containing aglycon, which generally taste bitter. Intact cyanogenic glycosides are not toxic, but when hydrolysed by an intracellular enzyme β-glucosidase, produces toxic products such as hydrogen cyanide and some carbonyl compounds.

These compounds suppress natural respiration and cause cardiac arrest (Davis, 1991) Cyanide can alter glucose metabolism. Sadati et al. (2013) reported that cyanide exposure at the dose of 0.2 mg/L caused a significant increase in glucose concentration in common carp. Cyanide can also cause a reduction ATP/ADP ratio and shift towards anaerobic metabolism resulting in elevated lactate levels (Way, 1984). Sadati et al. (2013) reported increased activity of LDH enzyme in the serum, as a characteristic feature of lactic acidosis and an indication of anaerobic glycolysis when common carp exposed to a sublethal dose of cyanide (0.1-0.2 mg/l).

Gossypol: Gossypols are polyphenolic pigments, which are seen to be concentrated in the pigment glands of the genus Gossypium (cotton plant). Glandless cottonseed meal has significantly low gossypol (< 0.01%) but the glanded varieties contain about 1.3% of gossypol that appears to be toxic to fish. Gossypol also forms gossypol protein complex and may form deficiency of some amino acids like the 'methionine', which plays a role in fat metabolism. Gossypol reduces the bioavailability of another limiting amino acid, 'lysine'. Feeding formulated diets to fish containing appreciable amounts of gossypol, like cottonseed meal causes growth depression, intestinal and other internal organ abnormalities and anorexia. Gossypol reacts with iron and forms an inactive ferrous gossypolate complex. As a result, dietary iron can be successfully used to neutralise or counter the undesirable effects of gossypol in formulated fish feeds used for gossypol sensitive fish species.

Non-starch polysaccharides: Non-starch polysaccharides and oligosaccharides are present in a wide variety of plants such as grains, legumes and cereals. The principal oligosaccharides reported from soybean are sucrose, raffinose and stachyose. Pectin and cellulose are also non-starch polysaccharides which leads to a decreased nutrient utilisation of nutrients (Choct et al., 2010). High NSP in the feeds found to reduce the digestibility of fat by binding with minerals in the intestine (Storebakken et al., 1998). Sunflower, lupin and soybean contain pectins, galactans, cellulose and lignin. The diets containing the above substances have decreased feed intake and feed digestibility in hybrid striped bass and rainbow trout (Gallagher, 1994; Sanz et al., 1994).

Phytoestrogens: Non-steroidal estrogenic substances distributed in plants are known as phytoestrogens which present mostly in soybean, cottonseed, linseed and sunflower seed. Plant estrogens are chemically isoflavones that exist in the form of glycosides they can bind directly to estrogen receptors or convert into compounds that have estrogenic effects (Francis et al., 2001). Dietary estrogen has wide-ranging effects on many physiological processes in fishes like the induced vitellogenesis or an increase in plasma vitellogenin level (Kaushik et al. 1995).

Mimosine: It is an unusual amino acid, which affects the production of thyroxine and hence the growth of the organism. Although structurally resembles the amino acid, tyrosine, it functions as an antagonist to this amino acid. The deleterious properties of mimosine include, disruption of reproductive processes and teratogenic effects (D'Mello, 1991). Mimosine is reported from Leucaena leucocephala (ipil ipil) and contains about 3–5% of the total protein on a dry weight basis (Liener, 1989). This was used as alternative feed ingredients in fish feeds. Soaking in water was found to be effective in removing the mimosine content.

Azadirachtin: Azadirachtin is a triterpenoid, which is the main active component in the neem seed cake. Azadirachtin is concentrated in the kernel around 4-6 g of azadirachtin is found in 1 kg of seed. In neem, azadirachtin is considered as the main agent for controlling insects. It is soluble in polar organic solvents and slightly soluble in water (Morgan, 2009; Gopan et al., 2019a). Nisbet (2000) has detailed the antifeedant and toxic effects of azadirachtin in insects. A feeding deterrent or antifeedant has been defined as a chemical which inhibits feeding but does not kill the insect directly, the insect often remaining near the treated plant and possibly dying through starvation (Munakata, 1975; Morgan, 2009). The presence of azadirachtin hampers the utilisation of neem seed cake in livestock and aqua feeds, make it non-edible. Thus removal of this active principle is imperative to utilise the seed cakes. It is highly soluble in organic compounds; thus extracting with solvents is an effective method to remove it. Isolating protein from the seed cake also found to be adequate to remove the ANFs in the neem seed cakes (Gopan et al., 2019a).

Amelioration techniques: Several techniques are employed for ameliorating ANFs in plant-derived feed ingredients, which can be broadly classified as i) physical methods such as heating washing, pressure and dehulling. ii) Chemical methods include the use of alkali, acids, solvent, urea, ammonia, ions, salts etc. iii) Biotechnological techniques which include the application of microorganisms and genetics. Lastly, a combination of several techniques such as water soaking, heat treatment, fermentation, irradiation, supplementation of enzymes, essential amino acids and genetic modifications are used to ameliorate ANFs. Some of the commonly used amelioration processes are discussed below.

Soaking: It is an age-old practice used to eliminate ANFs from plant-derived feed ingredients. Soaking in water have been used by many researchers to improve the digestibility of the plant feed ingredients. Among the pre-processing methods to eliminate ANFs soaking was found to be effective method in many feedstuffs (Nwosu, 2010) Soaking can be done in a variety of solvents based on the solubility of the anti-nutrients to be removed. However, the major drawback is the nutrient leaching, i.e., loss of valuable nutrients in the soaking medium.

Extrusion cooking: It is another widely used method to remove anti-nutrients from plant-derived feed ingredients. During the extrusion process, high temperature (90-150°C) is applied along with pressure and moisture. Thus it eliminates all the heat-labile ANFs in the feedstuff along with other pathogens if present. At the same time, it improves the nutritional quality of the feedstuff by enhancing the digestibility. Extrusion treatment has been applied to improve the quality of some legume seeds such as soybean or rapeseed, alone or blended with peas.

Fermentation: It is an innovative approach by which nutrient digestibility bioavailability can be improved. Solid-state fermentation is mainly used for aquafeed ingredients. It is also an integral part of the food detoxification process, which significantly lowers the anti-nutritional content, also improves the nutritive value of food grains (Yuan et al., 2013). Fermentation of the oilseed meal with lactic acid bacteria resulted in a reduction of the tannin content, thus increased feed efficiency and contributed to enhanced growth response. Controlled fermentation encourages the multiplication of particular organisms and their metabolic activities in food. Apart from the inactivation of anti-nutrients, fermentation with probiotic organisms may contribute to bio-enrichment.

CONCLUSION

Quest for alternate feed ingredients to replace fish meal and fish oil addressed the need for utilising the plant-derived feed ingredients. Recently more efforts have been made in the field of utilising non-conventional feed ingredients to minimise the competition from others feeds producing sectors. As the presence of ANFs is the main factor which affects the utilisation of the non-edible plant seeds in the feed industry, it is the high time to remove it from the ingredient to improve their utilisation. In the case of conventional plant feedstuffs, the presence of anti-nutrients will not lead to mortality at the level they exist. However, it can produce an adverse effect on the fish but varies with different factors such as species, kind, level of the anti-nutrients and the culture systems. Hence, there is a need for a species-specific approach to investigate the effect of plant-derived anti-nutrients before employing effective elimination strategies.

REFERENCES

Abara, A.E., E.O. Udosen, and O.U. Eka. 2000. Estimation of calcium, zinc, hydrocyanate, oxalate and phytate in Dioscorea bulbifera tuber. Global Journal of Pure and Applied Sciences. 6(3): 449-454.

Choct, M., Y. Dersjant-Li, J. McLeish, and M. Peisker, 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australasian journal of animal sciences. 23(10): 1386-1398.

Davis, R.H. 1991. Cyanogens. In: D' Mello, J.P.F., Duffus, C.M., Duffus, J.H. (eds.). Toxic substances in crop plants. The Royal Society of Chemistry.

D'Mello, J.F., C.M. Duffus, and J.H. Duffus. 1991. Toxic substances in crop plants. Woodhead Publishing.

Drew, M.D., T.L. Borgeson, and D.L. Thiessen. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology. 138(2): 118-136.

FAO. 2009. The State of World Fisheries and Aquaculture, 2008. FAO, Rome, Italy.

FAO. 2016. The state of world fisheries and aquaculture. FAO, Rome, Italy.

FAO. 2018. The State of Food Security and Nutrition in the World 2018. Building Climate Resilience for Food Security and Nutrition. FAO, Rome, Italy.

Fenwick, G.R., K.R. Price, C. Tsukamota, and K. Okubo.1991. Saponins. In Toxic substances in crop plants. Edited by J.P. D'Mello, C.M. Diffins, and J.H. Duffus. The Royal Society of Chemistry, Cambridge, UK. pp. 285–327.

Francis, G., H.P. Makkar, and K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199(3-4): 197-227.

Gallagher, M.L. 1994. The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (Morone saxatilis× M. chrysops). Aquaculture. 126(1-2): 119-127.

Gopan, A., N.P. Sahu, T. Varghese, P. Sardar, and M.K. Maiti. 2019a. Karanj protein isolate prepared from karanj seed cake: Effect on growth, body composition and physiometabolic responses in Labeo rohita fingerlings. Aquaculture Nutrition. 26(3): 737-751.

Gopan, A., N.P. Sahu, T. Varghese, P. Sardar, S. Gupta, G. Gupta, and M.K. Maiti. 2019b. Preparation of Protein Isolate from Neem Seed: Biochemical Evaluation, Antinutrients and In Vitro Digestibility Study. Animal Nutrition and Feed Technology. 19(2): 203-216.

Hajra, A., A. Mazumder, A. Verma, D.P. Ganguly, B.P. Mohanty, and A.P. Sharma. 2013. Antinutritional factors in plant origin fish feed ingredients: the problems and probable remedies. Advances in Fish Research. 5: 193-202.

Kaushik, S.J., J.P. Cravedi, J.P. Lalles, J. Sumpter, B. Fauconneau, and M. Laroche. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 133(3-4): 257-274.

Libert, B. and V.R. Franceschi. 1987. Oxalate in crop plants. Journal of Agricultural and Food Chemistry. 35(6): 926-938.

Liener, I.E. 1980. Heat labile antinutritional factors. In: Advances in legume science, (Editors: R.J. Summerfield and A.H. Bunting), Kew London, Royal Botanic Gardens, pp: 157-170.

Makkar, H.P.S., 1993. Antinutritional factors in foods for livestock. BSAP Occasional Publication. 16: 69-85.

Makkar, H.P.S., G. Francis, and K. Becker, 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal. 1(9): 1371-1391.

Morgan, E.D. 2009. Azadirachtin, a scientific gold mine. Bioorganic & medicinal chemistry. 17(12): 4096-4105.

Munakata. K. 1975. *Azadirachta indica*: A Source of Znsect Feeding Znhibitors and Growth Regulators. Pure Applied Chemistry. 42: 57.

Nisbet, A.J. 2000. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. Anais da Sociedade Entomológica do Brasil. 29(4): 615-632.

Norton, G. 1991. Proteinase inhibitors. In Toxic substances in crop plants. Woodhead Publishing.

Richardson, N.L., D.A. Higgs, R.M. Beames, and J.R. McBride, 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). The Journal of nutrition. 115(5): 553-567.

Sadati, F., D. Shahsavani, and H. Baghshani, 2013. Biochemical alterations induced by sublethal cyanide exposure in common carp (*Cyprinus carpio*). Journal of Biological and Environmental Sciences. 7(20): 65-69.

Sanz, A., A.E. Morales, M. de la Higuera, and G. Cardenete. 1994. Sunflower meal compared with soybean meal as a partial substitute for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets: protein and energy utilization. Aquaculture. 128: 287-300.

Savage, G.P., L. Vanhanen, S.M. Mason, and A.B. Ross. 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. Journal of Food Composition and Analysis. 13(3): 201-206.

T. Storebakken, K.D. Shear, A.J. Roem. 1998. Availability of protein, phosphorus and other elements in fishmeal, soy-protein concentration and phytase treated soy-protein-concentrate-based diets to Atlantic salmon (Salmo salar). Aquaculture. 161: 365-379.

Way, J.L. 1984. Cyanide intoxication and its mechanism of antagonism. Annual Review of Pharmacology and Toxicology. 24(1): 451-481.

Yuan, L., Wu, L., Yang, C. and Lv, Q. 2013. Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. Journal of the Science of Food and Agriculture. 93(2): 254-261.