Alternative feed ingredients in aquaculture: Opportunities and challenges

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Abstract
Fish meal and fish oil are principal sources of protein and lipid in aqua diets around the world. Production of the fish meal and fish oil is significantly affected by sustainable issues of marine capture fisheries, variable climatic events and increasing prices of fish meal and fish oil. However, wide scale use of alternative feed ingredients in aquaculture industry enables sustainable aquaculture productions with limited dependency on fish meal. In this review opportunities and challenges of plant ingredients, insect ingredients, terrestrial animal by-products, microbial ingredients and genetically modified ingredients have discussed. In future, plant ingredients in aqua diets will continue to increase. Proper processing of terrestrial ingredients will ensure higher nutrient bioavailability. Genetically modified plant ingredients, insect meal, and microbial ingredients have higher potential in future aqua diets. Extensive research on the large-scale production of these ingredients and further studies on ingredient’s effect on fish health will ensure limited dependency on fishmeal in future aquaculture practices.

Keywords: Alternative ingredients, aquaculture, sustainability, plant ingredients, microbial ingredients, opportunities and challenges

1. Introduction
Over the past few years, global aquaculture production has continued to increase concurrent with declining capture fishery production. FAO statistics revealed that the share of aquaculture in total fish production increased from 13.4% in 1990 to 42.2% in 2012 [1]. Although this figure indicates that aquaculture has greater potential to meet the increasing demand for fish, supplying of feed ingredients for aquaculture is one of the major challenges. Aquaculture production totally depends upon the provision of nutrients as in other terrestrial farming practices [2]. Diets with proper nutrient balance are important in enhancing fish health and higher fish production. Global fish feed production was estimated to increase up to 70,969 thousand tons by 2020, which is a nearly 10-fold increase from 1995 [3]. With those escalating figures, there is an immense pressure on the fish feed ingredients that are used to produce the feeds. Fish meal and fish oil are the major ingredients in fish feeds. As fishmeal and fish oil are limited resources, those need to be replaced with novel feed ingredients in order to enhance the production [3].

1.1 Fish meal as an ingredient in fish diet
Feed manufacturing industry heavily depends on the fish meal as a dietary protein ingredient. Fish meal is an ideal resource to meet the essential amino acid requirement of the fish. The fish meal also has high protein content and good availability of micronutrients. On the other hand, fish oil contains some highly unsaturated fatty acids. Reduced usage of other meat and bone meals due to the Bovine spongiform encephalopathy/mad cow disease is another impetus for fish meal production. Global fish feed production was estimated to increase up to 70,969 thousand tons by 2020, which is a nearly 10-fold increase from 1995 [3]. With those escalating figures, there is an immense pressure on the fish feed ingredients that are used to produce the feeds. Fish meal and fish oil are the major ingredients in fish feeds. As fishmeal and fish oil are limited resources, those need to be replaced with novel feed ingredients in order to enhance the production [3].

Despite these available positives, fish meal and fish oil production are highly dependent upon the marine fishery productions and bycatch. Global fish meal production statistics revealed that production rates are declining an average of 1.7 per year [3, 7]. Extreme pressure upon the marine capture fishery productions and declining stocks also influenced the global fish meal and fish oil productions. On a global scale, a greater part of the fish meal is used in feed for terrestrial animals such as swine and poultry [4]. Consequently, increasing terrestrial animal...
production is competing for fish meal as a high-quality feed ingredient. Increasing competition and limited availability of fish meal and fish oil have motivated the aquaculture industry to find out new alternative feed ingredients.

2. Plant as an alternative feed ingredient

Incorporation of plant ingredients in the fish diet is widely used practice driven by higher abundance and lower price compared to the fish meal. A range of plant ingredients is used in aquaculture industry including grains (wheat and corn etc.), oils (soybean, sunflower, rapeseeds, cottonseed etc.), and pulses (beans, lupins, and peas etc.).

2.1 Challenges of plant ingredients in aquaculture

Imbalanced amino acid profile, lower protein content, and presence of anti-nutritional factors are several challenges associated with plant ingredients. “Anti-nutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals” [8]. All plants have phytochemicals for protection against predators. Therefore, use of plant ingredients in fish feed without proper treatments may cause significant challenges in fish health. Anti-nutritional factors have a different mode of actions but may have an adverse effect on feed intake and nutrient digestibility in fish species. An extensive review of major anti-nutrient factors has been presented by Francis et al. [9]. Several important plant anti-nutrients and their major effect upon fish has been presented in Table 1.

### Table 1: Key anti-nutritional factors and their effect on fish

<table>
<thead>
<tr>
<th>Anti-nutritional factor</th>
<th>Mode of action</th>
<th>Effect on the fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease inhibitor (Kunitz soybean trypsin inhibitor and Bowman-Birk protease inhibitor)</td>
<td>Making stable complex with trypsin and reduce the activity of trypsin; Stimulate the secretion of cholecystokinin (cck) hormone and trypsin from gut wall and pancreas respectively.</td>
<td>Reduce apparent digestibility of protein [10] and lipids in Atlantic salmon [9]; Growth retardation in Nile Tilapia (&gt;1.6 mg/kg) [11]; Growth retardation of rainbow trout [12].</td>
</tr>
<tr>
<td>Lectins</td>
<td>Binding to the glycated cell receptors in cell surfaces and reduce digestive processes, protein transport, and disturbing cell signaling pathways</td>
<td>Alter nutrient metabolism [13]; Reduced nutrient digestibility and inflammation at distal intestine in Atlantic salmon [14].</td>
</tr>
<tr>
<td>Saponins</td>
<td>Contain hydrophobic and hydrophilic segments that have an ability to form micelles when inserted into water; Increase the permeability of intestinal mucosal cells (enterocytes), inhibiting the nutrient transport (e.g. Gypsophylla saponins) and increased infiltration of different cells (e.g. macrophages, lymphocytes).</td>
<td>Damages to gill epithelium of fish [15]; Cause soybean enteritis (Intestinal inflammation) in salmonids at moderate to high inclusion level [16]; Lower growth performance in rainbow trout and Nile tilapia (at 1.1%-1.5% inclusion level) [9].</td>
</tr>
<tr>
<td>Phytates</td>
<td>Chelate the divalent and trivalent cations (e.g. Zn^{2+}, Mg^{2+}, Cu^{2+}) Formation of phytate-protein complex</td>
<td>Reduce bioavailability of minerals [17]; Damages in the pyloric caeca in chinook salmon Hypertrophy and vacuolization of cytoplasm of intestinal epithelium cells in carps.</td>
</tr>
<tr>
<td>Tannins</td>
<td>Binding with proteins, minerals and feed components</td>
<td>Reduce absorption of vitamin B_{12} [18]; Growth retardation in tilapia at 200 g kg(^{-1}) inclusion rate [19].</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>Glucosinolates combine with the myrosinase could produce secondary product (nitriles, thiocyanates) which are toxic to animals</td>
<td>Growth retardation and effect on the thyroid function of rainbow trout at 1.4-19.3 mmol kg(^{-1}) inclusion [20].</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Obstruct secretion of digestive enzymes and effect on movement of substrates in intestine</td>
<td>Decreased nutrient utilization and digestibility in trout [21].</td>
</tr>
</tbody>
</table>

The anti-nutritional factors can be grouped into heat labile and heat stable compounds. Anti-nutritional factors, such as lectins, protease inhibitors, and amylase inhibitors are heat labile proteins which are easily inactivated by heat. Phytic acids, phenols, and tannins are heat stable compounds. Heat stable compounds are typically non-destructible in heat processing and remain intact. Enzyme inhibiting anti-nutritional factors usually decrease secretion of relevant enzymes (e.g. protease inhibitors, lipase inhibitors) and responsible for higher secretion of pancreatic enzymes. As a result, digestion of protein, carbohydrate and lipids may be influenced. Ingredients processing methods such as solvent extraction are used to remove heat stable anti-nutrients. However, anti-nutritional factors, such as phytic acid will remain in the ingredient after solvent extraction.

Higher inclusions of plant ingredients in fish diet have a significant effect on fish health. These include immune response, stress and histological alterations. Enteritis is most common in Atlantic salmon after feeding with soybean meal. Enteritis is mainly occurred by anti-nutritional factor Saponin in soybean. However, more recent studies have addressed the effect of plant oil on the gut morphology of fish [22, 23]. Transcriptomic approach to assess the fish health after fed with plant ingredients has shown that full replacement of fish meal by plant diets usually alter the lipid metabolism, nitrogen metabolism and cause overexpression of hepatic genes [24].

Imbalance in the nutrient composition is another drawback in plant ingredients. This limitation is appearing in amino acid profile and the fatty acid profile in plant ingredients. Amino acid profile of plant ingredients is not totally compensated to meet the EAA (Essential Amino Acid) requirement of fish (e.g. soybean; high in arginine and low in lysine). This leads to the combined use of one or more plant ingredients to correct balance of the AA profile of fish. One major example is a mixture of corn gluten (high in methionine but low in arginine and lysine) and soybean (high in arginine and lysine, low in methionine) meal to compensate the deficient AA requirement. However, these kinds of combinations could be challenged by the interaction of different anti-nutritional factors in plant ingredients.

Fatty acids in plant ingredients are devoid of HUFA (Highly Unsaturated Fatty Acid), especially in DHA and EPA. Full
replacement of fish oil with plant oil may cause a deficiency in essential fatty acid profile in fish. In contrast to that, plant oils are rich in medium chain triglycerols (MCT) that increase the performance of fish [9]. But higher inclusions usually increase the fish mortality [9]. Plant oil such as palm oil contains a higher amount of saturated fatty acids that leads to lower digestibility of energy at low water temperatures. This limits the widespread use of plant oils, especially in cold water aquaculture.

Ingredients processing techniques have a significant impact on the availability of EAA and other major nutrients in plant proteins. Most of the plant protein ingredients are by-products of residual from the industrial manufacturing process of vegetable oil and starch [25]. Those are subject to various processing techniques including heat treatment and solvent extraction etc. These processes will lead to denaturing the available proteins, oxidation, and binding with nutrients which may reduce the bioavailability of EAA to fish. Other challenges in the plant ingredients also include the presence of components such as mycotoxins and environmental pollutants. Mycotoxins are prevalent in plant diets including grains (maize, cottonseed and other grains etc.). Frequent monitoring programs, proper storage of ingredients and risk assessments are needed to avoid contaminations of plant ingredients with these external toxins.

2.2 Opportunities of plant ingredients

Although there are several undesirable characteristics, the value of plant ingredients in aquaculture practices is innumerable. Major obstacles in plant ingredients can be achieved by application of the various level of technology. For an example, higher protein content in soy protein concentrates (e.g. soybean, corn gluten meal) is achieved by removing the carbohydrate fraction. Treatment of both heat labile and heat stable anti-nutritional compounds of plant ingredients can be achieved by extrusion, heat treatment and fractionating of the crops respectively [26]. The fractionating process of plant feed ingredients includes simple methods such as de-hulling of crops and more advanced methods such as solvent purification. Enzymatic treatment of plant ingredients (e.g. phytates remove by phytase) enhances the nutritional quality of plant feed. Fish fed with these treated products have shown improved feed intake, higher growth [27], increased phosphorous and crude protein digestibility [28]. Plant oils are widely used in aquaculture since last decade due to its lower cost and higher availability. Soybean, palm, rapeseed and sunflower oil are the major ingredients that use for production of plant oils [29]. As a partial substitution to the fish oil, soybean oil, linseed oil and palm oil have shown positive results. Soybean oil and rapeseed oil contain a higher amount of PUFA (Poly Unsaturated Fatty Acid) (oleic and linoleic acids) which is required by the fish. Plant oil is sustainable in terms of water requirement of the oil-producing crops [30]. Wide-scale cultivation of these crops can be done in semi-arid/ arid regions with minimum water requirement. In addition to that increased biofuel/ethanol production from plants also produce different co-products. Those co-products can be used in aquaculture practices with further processing. Some of these co-products (e.g. dried distilleries grain) is not included anti-nutritional factors and contain moderate protein level. In an environmental perspective, low phosphorus level of these dried distilleries-grains leaves less ecological footprint [31]. Table 2 summarizes the challenges and opportunities major plant ingredients.

<table>
<thead>
<tr>
<th>Plant ingredient</th>
<th>Challenges</th>
<th>Opportunities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td>Contains heat stable anti-nutritional factors e.g. Saponins, phytic acid</td>
<td>Favorable EAA profile and high content of protein</td>
</tr>
<tr>
<td></td>
<td>Some of the processing methods (e.g. Solvent extraction) leaves the anti-nutrient compounds still in the meal</td>
<td>Ability to produce soy protein concentrate (low in anti-nutritional factors, soluble carbohydrates)</td>
</tr>
<tr>
<td>Pulses</td>
<td>Higher carbohydrate content than protein</td>
<td>Ability to produce protein concentrates which can increase the protein amount</td>
</tr>
<tr>
<td>Wheat gluten meals</td>
<td>Presence of anti-nutritional factors especially phytic acid and tannins</td>
<td>Contains 85% of protein in dry matter basis</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>Deficiency in lysine, Methionine, and Arginine</td>
<td>Higher digestibility in Atlantic salmon, Rainbow trout, and Coho salmon</td>
</tr>
<tr>
<td></td>
<td>Deficiency in lysine and arginine and high level of leucine</td>
<td>No morphological changes in distal intestine tissues of fish</td>
</tr>
<tr>
<td></td>
<td>Wet milling of corn gluten meal- leaves xanthophyll pigments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased production of genetically modified corn gluten meal</td>
<td></td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>Higher content of fiber</td>
<td>Ability to use against the salmon louse infection</td>
</tr>
<tr>
<td></td>
<td>Low digestive performance in fish due to high content of protease inhibitors, arginase inhibitors, and phytic acids</td>
<td>Preprocessing methods such as dehulling removes larger amount of fiber</td>
</tr>
<tr>
<td>Canola/ Rapeseed</td>
<td>Contains higher amount of fiber, glucosinolates</td>
<td>Ability to produce canola protein concentrate by aqueous extraction of fiber (higher protein content)</td>
</tr>
<tr>
<td></td>
<td>Presence of anti-nutritional factors specially phytic acids, glucosinolates</td>
<td>Reasonable level of linolenic acid and low level of linoleic acid in rapeseed oil trigger the own production of EPA and DHA in salmonids at reasonable level</td>
</tr>
<tr>
<td>Lupins</td>
<td>Deficiency in methionine and lysine</td>
<td>High content of arginine and glutamic acid contains a low level of anti-nutritional factors</td>
</tr>
</tbody>
</table>

3. Insects as potential feed ingredient

Use of insect as fish feed ingredients is quite novel to the aquaculture sector. However, a wide range of insect species is currently used in aquaculture practices. This includes rat tail maggots (Musca domestica), black soldier flies (Hermetia illucens), silkworm pupae (Bombyx mori), Grasshoppers, termites and mealworms (Tenebrio molitor etc.). Broader scope in availability (availability over seven taxa), rich in protein...
content and favorable lipid profiles make insects as ideal candidates for replacing fish meal. Replacement of fish meal with insect diet primarily depends upon the nutritional profile of insects. The protein contents in insects’ diets vary from 50-82% (dry matter basis) which is in the same range as fishmeal [32]. Insect meal is rich with EAA (lysine and methionine) although some minor variations are visible depending upon the taxon. Insect meal also contains compounds such as taurine and hydroxyproline which are deficient in plant diets [32]. Lipid composition of the insects contain a higher amount of polyunsaturated fatty acids (PUFA) n-6 than fish meal [33]. Various studies have reported positive results when fish were fed with insect meal in the diet. Increased growth rate and higher protein efficiency ratio have been recorded in fish fed with Z. variegatus [33]. Increased antioxidant activity [34] and recovery from lesions by improving the hematological parameters (red blood cells, white blood cells) also recorded when fish were fed with insect diets [35]. However, results may vary with fish/insect species, inclusion rate, and processing methods.

In production perspective, insect meal can readily be produced in farm facilities. It requires no land/water environment for productions. Most of the insect larvae can grow on poultry and livestock waste. Insects growing on these substrates could convert farm waste into organic manure. Insects also have an ability to reduce the pathogenic load in farm waste. In an environmental perspective, low emission of carbon can be achieved by biodegradation activity by insects.

3.1 Challenges of insects as fish meal substitute
The exoskeleton of the insects is made of polysaccharide chitin. Chitin in insects particularly remains indigestible in most of the fishes despite the availability of chitinase enzyme. Bioaccumulation of pesticides through insects in fish has been also reported [36]. In terms of fatty acids, low amount of PUFA in terrestrial insects reduces its suitability as marine fish feed. Mass production of insects for aquaculture practices is still in developing stage. Therefore, future studies should be more focused on technological improvements to enhance insect productions and understand the effect of insect meal on fish health.

4. Terrestrial animal by-products
Animal by-products include blood meal, hydrolyzed feather meal and poultry by-product meal etc. These rendered animal by-products have numerous advantages in aquaculture practices. Terrestrial animal by-products are free from anti-nutritional compounds.

4.1 Blood meal
Clean and fresh animal blood is used in the production of blood meal. Due to the strict regulations and safety concerns, only non-ruminant blood is currently used in aquaculture industry (especially in Europe). Crude protein level and crude fat level of blood meal usually reach approximately 85% and 0.5%-3% respectively [37]. Blood meal has favorable content of lysine and histidine. Inclusion rates of blood meal vary according to the life stage of the species of concern. However, the inclusion of blood meal at the rate of 5-10% may gain optimal results [38]. Consistent quality and higher digestibility have driven wide-scale use of blood meal in aquaculture [39]. Blood meal can also prevent cataract in cultured salmon [40].

4.1.1 Challenges of blood meal in aqua-diets
Processing method has a significant effect on the digestibility of blood meal. An Inverse relationship between heat application and lysine availability of blood meal was also reported [41]. Increased heat application usually deteriorates hemoglobin and cause low palatability. Blood meal produced by spray drying has shown higher digestibility than other methods [39].

4.1.2 Feather meal
Feather meal usually derives as a by-product from the poultry industry. Use of feather meal as partial or full replacement of fish meal has been experimented over several fish species including Labeo rohita [42], Oreochromis niloticus [43], rainbow trout [44] and other teleost species. Inclusion level of feather meal in fish feed diets is ranging from 50%-100% (depend upon species). Higher inclusion rates of feather meal in fish diets are depending upon production technology [45, 46]. However, at higher inclusion rates feather meal showed impairment of growth in several fish species [42, 43]. From a nutritional point of view, feather meal is low in certain essential amino acids including lysine, methionine, and histidine [38]. However, supplementation of these essential amino acids along with feather meal could enhance the growth of fish. In contrast to that, the presence of antimicrobial residues in feather meal could accumulate through feeding practices [47].

4.1.3 Poultry by-product meal
Poultry by-products consist of ground parts of poultry waste including legs, necks, underdeveloped eggs, and a limited amount of feathers which are unavoidable in processing practices. Poultry by-products are considered as a cost-effective feed ingredient in aqua diets. However, challenges of poultry by-products include a low level of available EAA (lysine, methionine, and histidine), the effect on nutrient availability by processing technology (e.g. heat drying), variable level of digestibility and impaired growth rates at higher inclusion levels.

5. Microbial ingredients for fish meal replacement
Over the past decade, use of microbial feed ingredients in aquaculture practices has gained wider attention. Microbial ingredients primarily include bacteria, microalgae, and yeast. Most of these microbial ingredients can be produced by waste treatment or obtain as a by-product of refinery processes. Methanotrophic bacteria can be grown in a larger amount with minimum dependence on soil, water, and climatic conditions [48]. Yeast can be obtained as a by-product of brewery plants and agricultural waste fermentation process. The bacterial meal has compatible protein and lipid content as fish meal [49]. Amino acid profile of bacterial meal is high in tryptophan but lower in lysine compared to the fish meal. Lipid profile of bacterial meal consisted of phospholipids. The fatty acid profile is dominated by C 16:0 and C 16:1 n-7 [23, 48]. The Vitamin content is dominated by vitamin B [48]. Recent studies have shown promising results when methanotrophic bacteria used as a fish meal replacement. These include increased growth efficiency, feed conversion rate, and improved gut health in Atlantic salmon and rainbow trout [48]. In terms of gut health, the bacterial meal can be helpful in recovering soy meal-induced enteritis in salmon. Moreover, the bacterial meal has proven capability to reduce expression of genes associated with inflammatory response in distal intestine of fish [48].
Yeast is a single cell organism, widely used in various industries including brewery and bakery. Dietary yeast can enhance the gut health of fish species as a probiotic and it also bears a number of nutritional properties. The crude protein content of yeast ranges between 42-55% and has shown higher digestibility in farmed fish [50]. Also, Dietary yeast can act as an immunostimulant, and boosting agent in Atlantic salmon reliving the soybean meal induced enteritis.

Microalgae as a potential feed ingredient in aquaculture practices have shown a compatible nutritional profile as fish meal. Various microalgal species (e.g. *Chlorella sp.*, *Scenedesmus sp.*, *spirulina sp.*, *Dunaliella sp.*) are used in aquaculture. Nutritional composition of the microalgae may vary with the species. However extensive review over seven major classes on microalgae has shown that there is no significant variation in amino acid composition among microalgal classes [51]. Most algae in this review showed tolerable lipid composition containing a high amount of n-3 PUFA, EPA and DHA [51].

Potential benefits of the microalgal products have been recorded over various fish species. These include, effect on the growth of fish (depends upon inclusion level), the effect on skin coloration, improved carcass quality, diseases and stress tolerance [52-54]. Astaxanthin is widely used as a source of pigment in salmonid aquaculture. Synthetic astaxanthin pigments in commercial aquaculture can be replaced by natural algae pigments obtained from *Haematococcus Pluvialis* [55].

### 5.1 Challenges of microbial feed ingredients

Microbial feed ingredients are faced with several challenges. Most of the microalgal products contain rigid cell walls that limit nutrient bioavailability. Rigid cell walls are also characteristic in bacterial ingredients and yeast. Although a number of processing methods are available to solve this issue, some of the processing methods (e.g. pressure application) are incapable of improving the bioavailability of the nutrients [49]. Commercial production of microbial ingredients usually associated with higher production cost. This cost involves designing effective culturing systems, harvesting technologies and feed development in microalgae. Large-scale production of methanotrophic bacteria also involves higher cost when using methane gas as substrate.

### 6. Genetically modified ingredients

In recent years, developments in the genetic engineering have shifted the conventional farming practices of vegetables and other farming crops to gain higher yield. Genetically modified plant ingredients have altered genetic material compared with their natural counterparts [56]. Statistics have shown that production of Genetically Modified (GM) crops is increasing by 4% in each year [57]. Inevitably, this increment has urged the feed manufacturing industry to use GM plant ingredients in feed manufacturing process.

GM plant ingredients have more favorable characteristics such as disease/ pest resistance, higher nutritional quality, longer shelf life, and free from anti-nutritional factors. Recent advances in recombinant DNA technology have increased the EAA profiles in several crops. Increased lysine profile in GM maize was achieved by incorporating *Corynobacterium glutamicum* genes [58]. Higher methionine profile in GM lupins was achieved by incorporating sunflower origin methionine gene [59].

GM plant ingredients in world market include GM-soybean (RRS®), RR canola, GM-maize (MON 810) and GM-lupins etc. A number of studies have been conducted to understand the effects of GM ingredients on immunological changes, histopathological changes, digestibility and expression of transgenic DNA in fish. (Table 3). These studies have shown various results depending on inclusion level, species, duration of the experiment and ingredient of concern. The basic concern of GM ingredients is an expression of the modified gene in targeted species. However, there is no solid evidence on horizontal gene transfer from GM plant ingredients to the fish or gut-associated microflora [60]. Most of the times, feed preparation processes (extrusion, heat treatment) destroy the inserted DNA fragments of GM ingredients. DNA fragments that sustain in feed preparation processes may be degraded by intestinal chyme of the fish. However, foreign DNA fragments that are passed through the feed preparation process and intact in enzymatic digestion may be available in other organs of fish (e.g. spleen, liver). These DNA fragments are below the level of coding functional protein in fish [61].

### Table 3: GM feed ingredients and main effects on fish

<table>
<thead>
<tr>
<th>Criterion</th>
<th>GM plant</th>
<th>Fish species</th>
<th>Inclusion%</th>
<th>Duration</th>
<th>Main effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of transgenic DNA</td>
<td>RRS®</td>
<td>Atlantic Salmon</td>
<td>17</td>
<td>6 weeks</td>
<td>No transgenic expression of gene in liver, brain and fish tissues [62]</td>
</tr>
<tr>
<td></td>
<td>BT maize</td>
<td>Atlantic salmon</td>
<td>30</td>
<td>82 days</td>
<td>Some differences in regulation of selected genes in the liver [63]</td>
</tr>
<tr>
<td>Histopathological changes</td>
<td>RRS®</td>
<td>Atlantic salmon</td>
<td>17</td>
<td>3 months</td>
<td>Higher inflammation in distal intestine [64]</td>
</tr>
<tr>
<td>Growth performance</td>
<td>Maize MON810®</td>
<td>Atlantic salmon</td>
<td>15-30</td>
<td>82 days</td>
<td>Reduced feed intake, reduced growth performance [65]</td>
</tr>
<tr>
<td></td>
<td>Maize MON810®</td>
<td>Zebrafish</td>
<td>20</td>
<td>3 weeks</td>
<td>Increment of growth [66]</td>
</tr>
</tbody>
</table>

### 6.1 Challenges of GM plant ingredients as potential feed ingredients

Use of GM plant ingredients in aquaculture industry is in it’s earliest stage. The inclusion of the GM plant ingredients is also strictly regulated by the certain parts of the world, especially in Europe. According to EU legislation (1829/2003 and 1830/2003), any feed material which is exceeded 9g/kg of GM material should be labeled [62]. Although the nutritional composition of plant ingredients has been improved by gene modification, negative effects on nutrient balance and growth rate are also visible in fish fed with GM diets [60]. On the other hand, consumers are skeptical about consuming fish fed with GM ingredients. Further researches on anti-nutritional aspects of GM plant ingredients and possible expression of transgenic DNA in fish are needed for careful evaluation of GM plant ingredients.

### 7. Conclusion

Fish feed production is in the process of rapid development. Feasibility of novel feed ingredients in aqua diets has been
studied in several studies. Findings of these studies have shown both positive and negative results. Further studies on the effect on fish health, improvements in legislation, and ensuring the consumer trust are essential for the successful transition from fish meal to alternative ingredients.

8. Acknowledgements

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