Hindawi
Aquaculture Research
Volume 2023, Article ID 2633412, 16 pages
https://doi.org/10.1155/2023/2633412

Review Article

A Review on the Use of Azolla Meal as a Feed Ingredient in Aquatic Animals' Diets

Mpwaga Alatwinusa Yohana,[1,2,3] Gyan Watson Ray,[1,2,3] Qihui Yang ,[1,2,3] Beiping Tan,[1,2,3] Shuyan Chi,[1,2,3] Amos Asase,[4] Kou Shiyu,[1,2,3] and Asare Derrick [1,2,3]

[1]*College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China*
[2]*Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang 524088, China*
[3]*Aquatic Animals Precision Nutrition and High-Efficiency Feed Engineering Research Center of Guangdong Province, Guangdong Ocean University, No.1 Haida Road, Mazhang District, Zhanjiang, Guangdong, China*
[4]*Department of Fisheries and Water Resources, University of Energy and Natural Resources, Box 214, Sunyani, Ghana*

Correspondence should be addressed to Qihui Yang; qihuiyang03@163.com

Received 16 March 2023; Revised 31 July 2023; Accepted 24 August 2023; Published 4 November 2023

Academic Editor: Wing-Keong Ng

The aquaculture industry's insatiable demand for fishmeal has led to a price surge, prompting the search for a sustainable alternative protein source. Enter Azolla meal, a plant protein that could be a viable substitute for fishmeal. This cost-effective supplemental feed could be a game-changer for small-scale producers looking to expand their aquaculture production. Numerous experiments have been conducted on replacing fishmeals with Azolla meals in fish diets, and the results have sparked further research. This review delves into Azolla's composition, comparing it to fishmeal in terms of proteins, amino acids, fatty acids, vitamins, and minerals. It also explores the value of employing Azolla in fish feeds and the various methods to mitigate its adverse effects through feed additives during the feed formulation. This review discusses the essential effects of Azolla meal on the growth of various fish species, including tilapia (*Oreochromis niloticus*), catfish (*Clarias batrachus*), thai silver barb (*Barbonymus gonionotus*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), and catla (*Catla catla*) and mrigal (*Cirrhinus cirrhosus*). Azolla's potential extends beyond its use as a fish feed ingredient. It could also be utilized for bioremediation to remediate hazardous chemicals in water resulting from aquaculture, industrial, and household waste. Further research is needed to optimize the use of Azolla meal in fish feed and to develop cost-effective production methods. This review is a valuable resource for farmers looking to maximize profit when using Azolla in aquafeed and potentially sustainably enhance aquaculture production. With its promising potential, the Azolla meal could be the key to meeting the growing demand for fishmeals while reducing the industry's environmental impact.

1. Introduction

Aquaculture is a rapidly growing industry that provides animal food, and it is worth considering as a viable alternative to other sectors. However, it is important to remember that sustainable development in aquaculture depends on various factors, including affordable feed. The economics of fish farmers will directly benefit from the efforts of feed formulators to prepare feed at a lower cost.

Fish nutrition is a crucial aspect of aquaculture, as it determines the health and development of fish [1]. The nutritional content of feed varies depending on the species and life stage of the fish being farmed. Fish require a variety of nutrients, including significant amounts of protein [2]. The protein ingredients used in aquafeed must meet several key criteria, including an excellent amino acid profile, high digestibility, good palatability, and no antinutritional components. They must also be available reliably and at a reasonable price [3].

Fishmeal is the primary protein source used in aquatic feed formulation because it is nutrient-rich. It contains essential amino acids (EAAs), minerals, vitamins (choline, biotin, and vitamin B12), A, D, and E, and omega ($n-3$) fatty acids. Due to its essential benefits, fishmeal is mainly used for livestock, poultry, and aquatic animal feed [4]. However, the overreliance on fishmeal has led to a decrease in wild fish populations, as wild fish are used as raw material in producing fishmeal [5, 6]. In addition, the rising cost of raw materials, such as fishmeal, has restrained the growth of aquaculture. According to Alfiko et al. [7], aquafeed accounts for a staggering 60%–70% of the overall cost of aquaculture output. As such, alternative protein sources in aquafeed have become a hotly debated topic in the industry. With the high cost and limited availability of traditional animal proteins like fishmeal, feed formulations have shifted toward plant-based proteins [8, 9]. Plant proteins such as soybean meal, wheat gluten meal, cotton meal, and distillers dried grain with solubles have been found to be effective substitutes for fishmeal in fish diets [10, 11]. However, the growing demand for plant-based proteins in human food and global trade has put pressure on supply and demand, leading to increased prices and concerns about environmental sustainability [12]. To address these challenges, researchers have turned to water weeds as a potential alternative feed source for aquaculture. Water weeds, which have traditionally been considered waste, can be used as feed ingredients, accelerating the creation of new aquaculture feed ingredients [13]. One promising variety of aquatic weeds is Azolla.

Azolla is a floating aquatic plant initially used as green manure but has since been used as a mosquito repellent, herbicide, water purifier, and nitrogen fertilizer for rice [14]. Azolla meals are a potential source of protein for fish, and their use in aquafeed could help reduce costs and increase profitability in fish farming operations [15]. Azolla has been used for centuries as a natural fertilizer for rice paddies in Asia, including China and Vietnam [16]. Its symbiotic relationship with cyanobacteria allows for the fixation of atmospheric nitrogen [17, 18, 19], which is then transferred to the soil when the plants are incorporated into the rice fields [20]. In recent years, Azolla has gained attention as a potential feed source for aquaculture due to its high protein content and ability to grow rapidly.

Scientific studies have shown that Azolla meals can be used directly as a food component, dried, or mixed with other commercial feeds to feed fish. Its high crude protein content, EAAs, vitamins, and carotenoids promote growth performance and immunity in fish [21–23]. Several aquatic animals species, including common carp, tilapia [24, 25], rohu [26], and freshwater prawn (*Macrobrachium rosenbergii*) [27] have been fed with Azolla-based diets, resulting in improved growth and health. Despite its potential benefits, the economic feasibility of Azolla production and its drawbacks, such as potential contamination with heavy metals and pesticides, need to be considered. Nevertheless, Azolla has the potential to be a sustainable and cost-effective alternative to fishmeal in aquaculture.

This review aims to provide a comprehensive overview of the nutritional value of the Azolla meal and its potential in various fish species. It will also assess the economic feasibility of its production and discuss the potential benefits and drawbacks of using Azolla meal as a fish feed. In this review, we will delve into the impact of Azolla meal on fish growth, health, and the environment. We recognize that using Azolla meal in aquaculture has benefits and challenges, and we aim to provide solutions to mitigate any negative effects. One approach we will discuss is the addition of feed additives during feed preparation, which can improve the quality of Azolla meals and promote fish health and growth. By exploring these solutions, we hope to contribute to the development of more sustainable and responsible aquaculture practices. Our discussion will greatly interest aquaculture practitioners, researchers, and policymakers committed to promoting eco-friendly practices in the industry. We believe that by addressing the challenges and benefits of using Azolla meal, we can help to create a more sustainable future for aquaculture.

2. Chemical Composition of Azolla Plant

Azolla has a unique chemical composition that makes it a remarkable organism with various applications. Azolla contains a high protein concentration, ranging from 25% to 35% of its dry weight [28, 29]. Furthermore, Azolla contains EAAs such as lysine, methionine, cystine, threonine, tryptophan, arginine, isoleucine, leucine, phenylalanine, tyrosine, glycine, serine, and valine [30, 31], which are higher than soybean meal except for histidine [32]. Azolla is also rich in minerals such as calcium, phosphorus, potassium, zinc, cobalt, chromium, boron, nickel, lead, cadmium, iron, manganese, and copper. It contains biopolymers and probiotics [33]; its low acid and neutral detergent fiber (NDF) suggest improved animal consumption [34]. Apart from being a plant protein source, Azolla is also a source of pro-vitamin A and contains chlorophyll, carotenoids, and growth promoter intermediaries [35, 36]. The body coloration of fish is influenced by their diet, specifically by carotenoids [37]. The consumption of carotenoid-rich food sources directly affects the availability and accumulation of these pigments in an organism's tissues, resulting in vibrant and attractive coloration. Research has shown that Azolla contains between 206 and 619 mg/kg of carotene, which makes it an excellent source of this essential nutrient [23, 38]. The amount of carotene in Azolla increases when the plant is grown under nutrient-rich conditions, making it an ideal aquaculture plant.

In addition, Azolla positively impacts water quality, reducing the need for chemical treatments and improving overall ecosystem health. Azolla's nitrogen-fixing capacity makes it an excellent biofertilizer for rice production, adding manure to the paddle soil [39]. The amino acid compositions are presented in Table 1 below, while the chemical compositions of Azolla can be found in Table 2. These unique properties make Azolla a versatile organism with applications in various fields. From being a source of nutritious food and livestock feed to serving as a biofertilizer and phytoremediator, Azolla contributes to sustainable agriculture and environmental remediation.

3. The Growing Conditions for the Azolla Plant

The Azolla plant is a fascinating aquatic fern that requires specific growing conditions to thrive. To cultivate this plant

Table 1: Amino acid composition of Azolla plant (*Azolla pinnata*).

Amino acid	Dry matter (%)	Protein (g/100 g)	Chemical score (%)
Lysine	0.98	4.58	130.9
Methionine	0.34	1.59	45.4
Cystine	0.18	0.84	24
Threonine	0.87	4.07	116.3
Tryptophan	0.39	1.82	52
Arginine	1.15	5.37	153.4
Isoleucine	0.93	4.35	124.3
Leucine	1.65	7.71	220.3
Phenylalanine	1.01	4.72	134.9
Tyrosine	0.68	3.18	90.9
Glycine	1.00	4.60	131.4
Serine	0.90	4.21	120.3
Valine	1.18	5.51	157.4

Source: Alalade and Iyayi [40].

Table 2: Chemical composition of Azolla meal on dry matter (%).

Index	Dry matter (%)
Crude protein	22.79
Crude fiber	15.49
Dry matter	90.3
Total ash	19.46
Ether extract	3.59
Nitrogen free extract	38.67
Calcium	2.03
Phosphorous	0.48

Source: Khursheed et al. [41].

Table 3: Nutrient concentrations in Azolla growth and the impact of organic and inorganic fertilizers on Azolla production.

Nutrients	Protein (g/m^2)	Increase over control (%)
Control	485	–
Cow manure	1,167	141
Compost fertilizer	1,131	133
Chemical fertilizer	811	67

successfully, it is essential to consider various factors such as temperature, humidity, sunlight exposure, soil acidity, and fertilization. According to Watanabe and Berja [42], the Azolla plant's growth rate, size, and tolerance to temperature and humidity vary. However, it grows best in stagnant water that is 5–12 cm deep and can be cultivated in ponds, containers, or pits. While the Azolla plant can tolerate a wide range of temperatures and humidity, excessive heat and humidity can slow down its multiplication and cause it to turn brown or reddish-pink. In addition, the plant cannot grow in acidic soil below 3.5. Therefore, it is crucial to monitor these parameters during cultivation.

The Azolla plant grows well in partial sunlight when under shade. However, excessive direct sunlight can cause stress and produce deoxyanthocyanins, which color the plant red. Interestingly, deoxyanthocyanins have antioxidative functions [43]. However, a high quantity of these compounds can deplete polyunsaturated fatty acids in the plant, reducing its nutritive value and palatability [44]. Adding fertilizer to the Azolla plant is advisable to promote optimal growth. However, it is crucial to supply the manure before putting it in the fern to ensure optimal phosphorus levels. A phosphorus deficiency can cause the plant to become smaller and turn pink to red. In addition, using an insecticide can prevent the spread of pests [45]. Finally, maintaining good water quality through regular partial water changes and keeping a check on dissolved oxygen levels is crucial for preventing bacterial infections and ensuring healthy growth. Overall, Table 3 below presents a summary of the impact of various fertilizers on Azolla production. The most significant yield of Azolla fodder was achieved by incorporating cow manure at a 1 kg/m^2 rate, producing 1,167 g/m^2. Following closely behind was compost, with a production of 1,131 g/m^2. Conversely, the utilization of chemical fertilizers resulted in the lowest production, yielding only 811 g/m^2. Table 4 shows the cultivation conditions for different species of Azolla plants.

4. The Steps Involved in Producing the Azolla Plant

Cultivating Azolla can be an effective way to supplement the diet of fish and improve their growth and overall health. To successfully cultivate Azolla, there are several important steps to consider. First, dig a pit according to the desired size, cover it with silpaulin sheets to make it waterproof, and remove any unwanted materials [59]. Second, spread fertile soil uniformly on the silpaulin sheets, pour water into the pit, and add fertilizer. Nutrients like nitrogen and phosphorus are crucial for Azolla's growth, so adding organic fertilizers or animal manure can help provide these essential nutrients to health [58]. Different types of manure can be used, such as fresh buffalo manure, vermicompost, or cow dung [60, 47, 51, 53, 61, 62]. The next step is to obtain Azolla spores or mother plants for propagation. Azolla

Table 4: Cultivation conditions for different species of Azolla plants.

Azolla plants	Temperature	Nutrient	Volume of water	pH	Salinity	Light intensity	Reference
Azolla pinnata, *Azolla filiculoides*	20–23°C	KNO_3, $KH_2PO_4Ca(NO_3)_2$, Fe, $ZnSO_4$, $MgSO_4$	1,505 cm^2	6–7	400–450 ppm	300 $\mu mol/m^2/s$	[46]
Azolla pinnata	18–28°C	Cow dung, phosphate, magnesium, iron, copper, sulfur	10 cm depth	4.5–7	–	Full to partial shade (100%–50% sunlight)	[47]
Azolla	20–30°C	Essential macro–micro nutrients, except for nitrogen	3–5 cm water depth	5–7	0.3%–2.5%	25%–50% of full sunlight for 20 hr/day (3,000–6,000 lux)	[43]
Azolla	–	P, Ca, Mg, and K	–	6.6–6.8	54.0 ppm	–	[48]
Azolla filiculoides	25°C	–	–	5.5	–	40–70 $\mu mol/s/m^2$ PPFD	[49]
Azolla pinnata and *Azolla filiculoides*	23–25°C	–	Surface area 130 cm^2	–	–	100 $mol/m^2/s$	[44]
circinata, *A. pinnata*, *A. rubra*, and *A. mexicana*	18–27°C	$CaCl_2$, K_2SO_4, $FeSO_4$, $ZnSO_4$, $MgSO_4$, $MnCl_2$	Surface area 340 cm^2	Neutral	–	15 klx	[50]
Azolla	–	Cow dung, phosphate	10 cm depth	–	–	–	[51]
Azolla	20–35°C	P, K, Fe	–	4.5–7.0	No more than 0.3%	20%–50% of full sunlight	[52]
Azolla pinnata	–	Cow dung phosphate and (urea) inorganic	10 cm depth	–	–	Partial shed	[53]
Azolla filiculoides	22°C		Surface area of 7,850 cm^2	6.4	370–430 ppm	20 LX	[54]
Azolla pinnata	–	Urea, K_2HPO_4	–	7.0	–	LX-101	[55]
Azolla filiculoides	15–20°C	–	–	4.5–7.0	–	–	[56]
Azolla micropylla	20–31°C	–	–	–	–	10,000 Lux	[57]

Source: Temmink et al. [58].

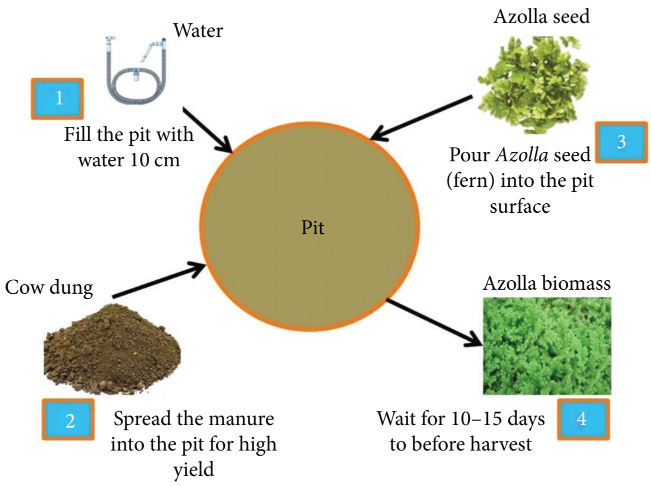

FIGURE 1: Process of cultivating Azolla plants.

reproduces through asexual reproduction, and its rapid growth enables it to cover the water surface in a short period [63]. The spores or entire plants can be obtained from specialized nurseries or other Azolla cultivators. These can then be spread on the water's surface, allowing them to multiply and form a dense mat of Azolla. Regular maintenance is necessary to ensure the optimal growth and productivity of Azolla. Monitoring the water temperature, pH, and nutrient levels regularly [64]. The plants should be harvested frequently, removing around 50%–70% of the biomass every 2–3 weeks. This helps maintain a healthy growth rate and prevents overcrowding, which can lead to reduced growth and suboptimal nutritional content. By following these steps, fish farmers can ensure a steady and reliable supply of Azolla to supplement their fish's diet. Integrating Azolla cultivation into aquaculture systems can improve the health and growth of the fish, contributing to sustainable and efficient fish farming practices. Figure 1 shows the process of cultivating Azolla plants.

5. The Impact of Using the Azolla Plant as a Fish Diet on Growth Performance

As fish grow, they increase in both length and weight. However, their growth is heavily influenced by various factors such as feed availability, oxygen levels, temperature, and water quality, including ammonia and pH. Unlike commercially reared animals, fish require a protein-rich diet to thrive. It is crucial to find the right amount of protein to promote growth and survival since it is the most expensive component in diet formulas. Fortunately, Azolla provides a better alternative to traditional fish diets due to its ideal protein and EAA content.

Azolla plants have been widely used as a feed ingredient for fish, including tilapia [25], redbelly tilapia (*Coptodon zillii*) [55], catfish [65], fringed-lipped carp (*Labeo fimbriatus*) [66], calbasu (*Labeo calbasu*) [67], and thai silver barb [53]. Researchers have conducted numerous studies on fish's growth performance and survival by feeding them raw or dry feed materials of Azolla plants [68]. These studies have shown that fry stage and fingerlings perform exceptionally well in terms of growth and survival when fed diets containing Azolla meal [67]. For instance, Magouz et al. [25] found that tilapia may consume up to 20% of Azolla meal, while Shiomi and Kitoh [69] found similar results. According to a study conducted by El-Sayed [68] and El-Sayed and Garling [70], young fish can be fed 100 g of Azolla meal, while adult tilapia can be fed up to 200 g. Other researchers have found that various freshwater fish diets can contain different percentages of Azolla meal. For example, rohu [71] can be fed 50%, thai silver barb 25%, and fringed-lipped peninsula carp 40% [66], and carp (*L. calbasu*) 30% [67]. Das and Rahim [53] conducted a study to investigate the effects of a 25% *Azolla pinnata* diet on the growth of thai silver barb. Surprisingly, their findings revealed no significant differences in the average growth rate, net production rate, and specific growth rate between the experimental group and the control group ($P > 0.05$). It is worth noting that no instances of mortality were observed within the fish treatment groups 2 and 3, as indicated in Table 5. Furthermore, the feed conversion ratio (FCR) was found to be low in fish fed with 25% Azolla, but increased as dietary Azolla levels increased. Similar results were reported in tilapia [73]. A lower FCR is an indicator of a higher protein efficiency ratio, apparent net protein utilization, apparent net lipid utilization, and apparent net energy utilization. Conversely, a higher FCR results in less tissue growth and more impurities in the water environment [74]. This means that nutrients in Azolla are converted into muscle while reserved protein, lipids, and energy are transferred into growth [75].

The digestibility of Azolla plays a significant role in determining its effectiveness as a feed. Fish require easily digestible nutrients to maximize their growth and overall health. The digestibility of Azolla, as shown in Figure 2, demonstrates its potential as a valuable feed source for fish. Moreover, the inclusion rate of Azolla in fish diets is crucial for achieving optimal productivity. The inclusion of Azolla in the right proportion can enhance fish growth and development, leading to increased productivity. High levels of Azolla inclusion can potentially weaken digestion and reduce feed consumption by impacting the activity of digestive enzymes [25, 76]. Several studies have been conducted to determine the digestibility of Azolla, and the results have shown promising outcomes. One study conducted by Magouz et al. [25] found that Azolla had a high digestibility rate in tilapia. This indicates that a significant portion of the Azolla consumed by the fish is efficiently broken down and absorbed, leading to better growth and overall health.

The combined findings of the earlier researchers demonstrated that feed could be prepared solely using plant-based protein sources without adding fishmeals. With the rising population, the demand for fish will increase if prices continue to rise at the current production rate. Therefore, exploring sustainable ways of using Azolla as fish feed is essential. We hope to inspire aquaculturists to conduct more research on the topic and report growth more precisely to obtain more information from the available data. In addition, examining which diet is best between raw and dried

Table 5: Showcases the growth performance of two fish species, carp (*Cyprinus carpio* var. *communis*) and thai silver barb (*Barbonymus gonionotus*), when fed with diets containing varying levels of Azolla meal.

Azolla diet (%)	Initial weight (g)	Final weight (g)	Survival (%)	Specific growth rate (%/day)	Feed conversion ratio	Protein efficiency ratio
Carp (*Cyprinus carpio* var. *communis*)						
$Diet_1$ (0)	3.51 ± 0.07	12.98 ± 0.41^a	100	1.16 ± 0.07^a	1.91 ± 0.75^d	1.50 ± 0.08^b
$Diet_2$ (10)	3.45 ± 0.06	12.48 ± 0.52^a	100	1.15 ± 0.05^a	1.75 ± 0.48^e	1.63 ± 0.06^a
$Diet_3$ (20)	3.45 ± 0.06	11.55 ± 0.56^b	100	1.07 ± 0.03^b	1.98 ± 0.39^d	1.44 ± 0.07^c
$Diet_4$ (30)	3.45 ± 0.06	10.64 ± 0.48^c	94	0.99 ± 0.04^b	2.40 ± 0.37^c	1.19 ± 0.09^d
$Diet_5$ (40)	3.45 ± 0.06	9.64 ± 0.63^d	92	0.91 ± 0.06^{bc}	2.60 ± 0.29^b	1.09 ± 0.05^e
$Diet_6$ (50)	3.45 ± 0.06	9.06 ± 0.47^d	91	0.87 ± 0.07^c	2.70 ± 0.36^a	1.05 ± 0.07^e
Thai silver barb (*Barbonymus gonionotus*)						
$Diet_1$ (0)	3.90 ± 0.13	30.93 ± 0.4^a	99.33 ± 1.15^a	3.70 ± 0.14^a	0.88 ± 0.09^a	2.98 ± 0.03^a
$Diet_2$ (25)	3.90 ± 0.11	30.68 ± 0.4^a	98.67 ± 1^a	3.68 ± 0.16^a	0.93 ± 0.17^a	2.94 ± 0.02^a
$Diet_3$ (50)	3.90 ± 0.29	24.55 ± 0.45^b	99.33 ± 0.58^a	3.28 ± 0.11^b	1.15 ± 0.12^b	2.26 ± 0.08^b
$Diet_4$ (75)	3.90 ± 0.09	19.81 ± 0.25^c	98 ± 1^a	2.90 ± 0.08^c	1.66 ± 0.15^c	1.75 ± 0.07^c
$Diet_5$ (100)	3.90 ± 0.08	15.20 ± 0.39^d	99.33 ± 0.58^a	2.43 ± 0.18^d	2.64 ± 0.06^d	1.24 ± 0.02^d

Source: Ahmed et al. [72] and Das et al. [53]. The significant differences between the control treatment group and the fish supplemented with Azolla meal are illustrated by a, b, c, d, e, and bc.

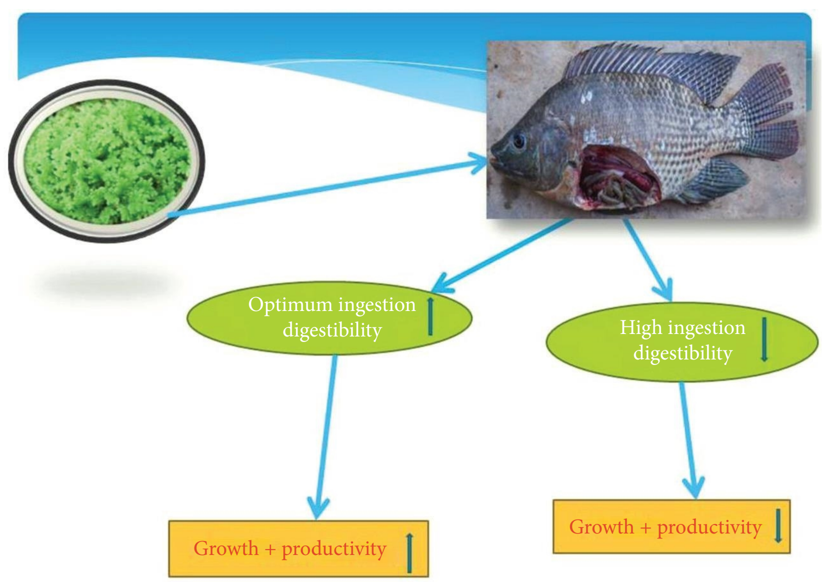

Figure 2: Highlights the digestibility of Azolla as a promising feed source for fish.

Azolla and which freshwater fish grow better after being fed with Azolla feed is crucial.

6. The Effects of the Azolla Plant in the Diet of Fish on Immunity

Dietary composition significantly affects how the immune system evolves and functions. The nutritional and chemical properties of the feed play a crucial role in this effect, as Mendivil [77] noted. In modern fish farming, the importance of maintaining fish health through ideal nutrition is widely recognized. Scientific data suggests that nutritional supplements and additives can boost fish immune systems and prevent infections [78]. High-quality diets are also marketed for their ability to promote health and prevent disease [79, 80]. Fish have immune systems similar to mammals and birds, as van Muiswinkel and. Vervoorn-Van der Wal [81] noted. They are a class of vertebrates and possess innate and adaptive immunity [82] and a sophisticated intrinsic defensive system that they use to defend themselves in their environment [83]. Fish are found in various aquatic habitats and effectively use their protection mechanisms in these conditions. However, due to the widespread use of aggressive culture techniques, the infection pressure is significantly higher in farms.

The innate response has been believed to be a crucial component in defending against infections in fish due to the limits of the adaptive immune system, the poikilothermic nature of fish, the limited repertoire of antibodies, and the delayed lymphocyte proliferation and memory [84]. The mucosal tissues connected to the fish's immune system include the skin, gills, and gut. The skin, in combination with its mucus, is regarded as the main barrier that offers physical and chemical protection. Animal epidermal mucus is a crucial defense mechanism against harmful bacteria. Innate immune substances found in this mucus, released by goblet cells, act as a barrier between the animal and its surrounding habitat [85]. Fish mucosal immune tissues also interact with and regulate the microbiota and beneficial microorganisms that inhabit mucosal surfaces, preventing pathogen infections [86].

Proper nutrition is essential to maintain the health of cultured fish and achieve optimal growth and immunity. The relationship between nutrition and immunology suggests optimal health results from a proper diet and feeding regimen. Supplementation of Azolla in fish diets improves intestinal health function, as it is high in EAAs, vitamins, minerals, and carotenoids, which help strengthen the immune and digestive systems [13]. Azolla's phenol content, carotenoids, flavonoids, and tannins have been linked to improving antioxidant and immunostimulant functions [87, 88]. Increased goblet cell production brought about by the addition of Azolla may promote immunity and disease resistance [25]. The mechanism of action includes aiding digestion and helping the intestinal goblet cells produce mucus and antimicrobial compounds that shield the mucosal barrier from infections, injury, and dehydration [89]. A study by Lumsangkul et al. [87] found that the biofloc system in both serum and skin mucus represented the immunological response of Nile Tilapia-fed dietary Azolla. The findings proved Azolla's intrinsic impact on intestinal immunity related to the body's immune system [87]. Fish fed 100 g/kg of Azolla under biofloc conditions displayed better growth performance due to serum and skin mucus immunity. The gills, which are primarily respiratory organs, contain lymphoid tissues that play a crucial role in the immune system's defense mechanism. Lymphoid cells refer to a group of cells that make up the immune system in fish, as explained by Bjørgen and Koppang [90]. Other lymphoid organs in fish include the kidneys, thymus, spleen, and gut-associated lymphoid tissues (GALT), which develop during the larval stage [91]. The gastrointestinal tract also plays a significant role in immunity and nutrition. Skin mucus samples taken 8 weeks after starting Azolla feeding exhibited greater lysozyme and peroxidase activities [87]. The mucus contains various components that support innate immunity, including antimicrobial peptides and lysozymes [92]. Lysozyme activity is one of the best indicators of a diet's bactericidal effect. It hydrolyzes the -1,4-glycosidic bond found in the polysaccharide layer of bacteria cell walls, making it particularly effective at killing Gram-positive bacteria [93]. When attached to microbes, lysozyme functions as an opsonization agent, stimulating phagocytosis [94]. The immune defense mechanism phagocytosis assists fish in reducing their chance of contracting infectious pathogens [95]. The findings supported the notion that Azolla inclusion can sustain immunological responses (lysozyme activity and phagocytosis) that contribute to pathogen resistance to tilapia [24, 96, 97], *Cyprinus carpio* [98]. It may be possible to use hematological and biochemical markers to identify the potential effects of dietary factors on the health of aquatic animals [33, 99]. Previous studies have shown that including Azolla in tilapia and common carp diets do not significantly affect their hematological and biochemical variables, such as hemoglobin, red and white cells, aspartate aminotransferase, and alanine aminotransferase [24, 100].

The digestive tract serves various purposes in teleosts, but defense is one of the most crucial. The GALT, which acts as a physical barrier to pathogen invasion, is present in the gut [101]. The growth and development of GALT, which mediates several host immunological activities, is critically dependent on gut microbes [102]. The lymphoid cells are dispersed throughout the mucosa in groups of immune cells such as mast cells, granulocytes, macrophages, and lymphocytes. These cells take up the antigen, digest it, and aid in developing immunological memory [103]. Defense cells, such as phagocytes, neutrophils, and macrophages, play a crucial role in the body's defense system [104]. During a respiratory burst, these cells primarily eliminate germs by creating reactive oxygen species.

Mishra et al. [14] demonstrated the nonharmful effect of Azolla inclusion in animal feeds. The findings show that the amount of Azolla included in the diet had no impact on the thymus and lymphoid organs for various treatment groups [105]. Heterophils are a type of white blood cell that play a crucial role in birds' immune systems. They are highly phagocytic, meaning they can engulf and destroy harmful bacteria. In fact, heterophils have a wide range of antibacterial abilities [106], making them an important line of defense against infections. Recent research suggests that adding dried Azolla to birds' diet does not negatively affect the levels of heterophils. This is great news for farmers, as it means that animals fed the Azolla diet had appropriate nutrition and did not exhibit any symptoms of anemia, infectious sickness, or parasite issues. However, the issue of lymphoid tissue in freshwater fish has yet to be addressed in the literature. Therefore, we propose that more research be conducted on fish lymphoid organs.

One measure of host health is disease resistance [24], which can be tested using bacterial challenges. Interestingly, Nile tilapia-fed dietary Azolla has been found to have a higher resistance to *Streptococcus agalactiae* infection [87]. This is likely due to the fact that fish-fed dietary Azolla have an active immune system, as evidenced by their elevated respiratory burst activity and greater resistance to bacterial infection [98]. Azolla has positive impacts on animals' immune systems, including fish. When the optimal amounts of inclusion were considered, the results demonstrated the significance of incorporating Azolla into animal immunity. In addition, the immune system's primary job is to fight disease by locating and eliminating infections. However, different factors such as stress, poor diet, infections, and external antigens can significantly impact immune function. Fish are also exposed to environmental and operational stressors throughout the production cycle, which can affect their ability to reproduce and the health of their immune systems.

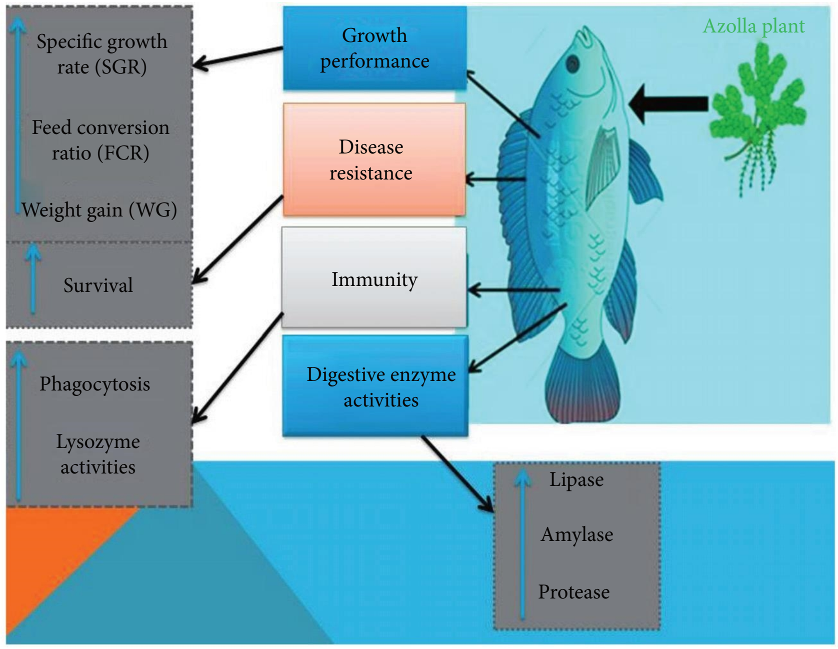

Figure 3: The effects of Azolla feed on growth performance, immunity, disease resistance, and digestive enzyme activities. The blue arrow shows increased parameters.

Therefore, it is essential to consider effective feed preparation to achieve the intended results for farmers and aquaculture growth generally. Immunostimulants can be administered to boost the immune system and improve disease resistance. Immunostimulants are essential for ensuring that fish's immune system is functioning optimally, thereby enhancing their growth performance. This, in turn, helps to prevent the negative consequences that can arise during stressful times. As depicted in Figure 3 below, Azolla can effectively improve growth performance and immunity in fish when utilized efficiently. By administering immunostimulants, fish farmers can rest assured that their fish are well-protected against diseases and other stressors that can impede their growth and development. Azolla plant has been shown to be particularly effective in enhancing fish's immune systems and promoting their growth. When used correctly, Azolla can help fish farmers achieve optimal growth rates and improve the overall health of their fish populations.

7. Challenges in Using the Azolla Plant as a Fish Feed

When using plant-based protein as feed ingredients for fish, it is crucial to analyze their growth performance, immunity, and mortality rate. However, it is equally important to consider the impact of amino acid imbalances on fish health caused by antinutritional factors (ANFs) present in plant ingredients. ANFs, such as protease inhibitors, phenolic compounds, phytates, lectins, and oligosaccharides, are present in variable amounts in plant protein sources, making it necessary to destroy them before adding them to aquafeeds [107]. The high concentration of nonsoluble carbohydrates like fiber and starch in plant ingredients makes them deficient in some EAAs, less palatable, and lowers nutrient digestibility [108, 109]. Fish are more susceptible to these antinutritional elements than terrestrial animals, making it crucial to eliminate them before adding them to aquafeeds. For instance, soybean's presence of ANFs induces an inflammatory response, reducing brush border and cytosolic enzyme activities and negatively impacting disease resistance [110]. The inflammation reduces brush border and cytosolic enzyme activities [111, 112].

However, some ANFs present in the plant protein feed make it harmful to the animals when consumed in large quantities. Reducing ANFs in plant feed is crucial for improving its nutritional value and increasing animal utilization. ANFs are naturally occurring compounds in plants that can negatively affect the health and productivity of farm animals by interfering with nutrient absorption or causing toxic effects [113]. However, implementing specific processes can minimize these ANFs, making plant feed safer and more nutritious for animals. Thermal processing is one of the most effective techniques for reducing ANFs [114]. Heat-treated methods such as cooking, toasting, steaming, or extrusion can break down or inactivate these harmful substances. For instance, cooking soybeans can reduce trypsin inhibitors [115], which hinder protein digestion, while extrusion processing can denature lectins, which impair carbohydrate absorption. These thermal treatments alter the structure and chemical composition of the feed, thereby enhancing its nutrient availability and digestibility for animals. Another important process for reducing ANFs is the use of chemical treatments [116]. In addition, chemical treatments may be used to neutralize or inactivate ANFs, such as tannins or protease inhibitors, to improve feed quality and animal performance. Azolla meals should be included in animal diets in moderate amounts and should be properly processed to reduce the levels of these harmful compounds. Increased Azolla meal levels in fish diets have been shown to reduce weight gain by increasing energy expenditure and decreasing digestibility, which is attributed to antinutritional elements [25]. A diet with many fibers is unsuitable for nonherbivorous fish species because they lack enzymes responsible for breaking down cell walls in the feed [117].

Azolla plants are known for their NDF, which is the cell wall of fern that is not digestible, especially for simple-stomached animals like fish. Buckingham et al. [118] found that NDF in Azolla plants can pose a challenge to the digestive system of fish. Shanab [55] observed that increasing the Azolla meal level by more than 20% resulted in poor development and weight loss in Nile tilapia. The nutrient composition of Azolla plants varies depending on cultivation methods, such as light intensity, temperature, humidity, and soil nutrients. These variations can affect the growth and contamination of epiphytic algae, which in turn affect amino acid composition [57]. Das et al. [53] reported that increasing the *A. pinnata* by more than 25% of the Azolla meal resulted in decreased growth rate of thai silver barb. Fasakin et al. [119] found that the poor growth of tilapia was due to a deficiency of EAAs such as tryptophan and threonine. Similarly, Fiogbé et al. [120] reported that fish fed with 20% low growth due to a deficiency of amino acids, which limits protein synthesis. When one amino acid is deficient, it restricts the intake of all other amino acids, regardless of quantity.

These challenges of poor amino acids in Azolla plants, the presence of NDF, and the presence of ANF such as phytate need to be addressed when adding Azolla to fish diets. Addressing these challenges will help reduce the negative impacts of Azolla on fish diets, leading to improved growth performance and immunity in fish. In addition, using feed additives such as enzymes and organic acids can help reduce the negative effects of ANFs in Azolla meals.

8. Solution for Limitations of Azolla Plant Use in the Aquaculture Industry

Improving and strengthening fish farming operations can be achieved through various measures, such as reducing fish loss, minimizing the period of increased growth, establishing a target for fish size, granting access to markets, and encouraging low-cost investment [121]. However, with the decline of fishmeal as a major source of protein in fish diets, aquaculture's future growth and sustainability may depend on discovering new, suitable, and less expensive alternative protein sources. While some studies have shown that feeding fish plant protein diets can have an adverse effect on their performance, plant-based protein can be used as an ingredient if the fish exhibit no variations in overall performance when fed a plant diet [111].

Several measures can be taken to mitigate the negative effects of Azolla meal utilization in aquaculture. It is crucial to ensure that the Azolla meal is of high quality and free from contaminants, achieved through proper cultivation and harvesting techniques and rigorous quality control measures. Balancing the amount of Azolla meal used in the feed with other essential nutrients required by the aquatic organisms is also essential to promote their growth and health. Regular monitoring of the growth and health of the aquatic organisms enables early detection of any negative effects of Azolla meal utilization and prompt corrective action. Several research findings have shown that feeding animals plant protein feed sustainably without causing harm to the fish can be achieved by incorporating certain dietary strategies, such as supplementing certain additives, applying exogenous enzymes, and adding deficient amino acids. By adopting these measures, aquaculture can continue to thrive sustainably and meet the growing demand for fish products. Incorporating probiotics into fish diets, particularly Bacillus species, can significantly impact gut health and stress relief [122]. Probiotics are a highly effective feed additive that can improve Azolla utilization in fish while also serving a variety of purposes, such as improving the intestinal shape and boosting fish immunity to minimize mortality rates [123, 124]. Research has been conducted on the use of probiotics, and Azolla in Patin fish (*Pangasius djambal*), which has shown that supplementing their diet with probiotics leads to good growth performance. However, when compared to controls and fish fed with pellets and probiotics, Patin fish supplemented with Azolla have even better growth and a more favorable profile of fatty acids [122].

The right enzymes must be present to ensure optimal digestion of fish feed and the absorption of necessary calories and vital nutrients [125]. Studies have shown that using phytase in aquaculture species can significantly impact growth performance, nutrients, and energy utilization [101]. Supplemental phytase can hydrolyze phytate, an indigestible form of phosphorus in plants, and increase the concentration of minerals ingested, including magnesium and calcium. Previous research has documented the effects of phytase on growth and feed utilization in Nile tilapia fed a plant-based diet [126]. By incorporating probiotics and enzymes into freshwater fish diets, aquaculture farmers can improve their fish's health and growth while reducing mortality rates and minimizing the environmental impact of aquaculture. Based on recent research, adding dietary phytase at a dose of 1 g/kg can significantly increase the nutritional value of soybean meal-based diets. This makes it possible to replace fishmeal entirely, which is a significant development in aquaculture. In addition, enzymes that enhance intestinal health and prevent pathogens have gained attention, which is crucial for managing and reducing antibiotic use. These enzymes have been used to reduce aquaculture diseases and promote the health of farmed fish. The most popular exogenous enzyme feed additives are glucose oxidase and lysozyme. Supplementing exogenous enzymes in plant protein feed is an effective strategy to enhance the digestibility and utilization of plant proteins in animal nutrition [127, 128]. By breaking down ANFs and improving nutrient availability, exogenous enzymes can improve animal performance and increase productivity.

Furthermore, organic acids play a crucial role in promoting gut health by creating an optimal environment for beneficial microorganisms [129], preventing the proliferation of

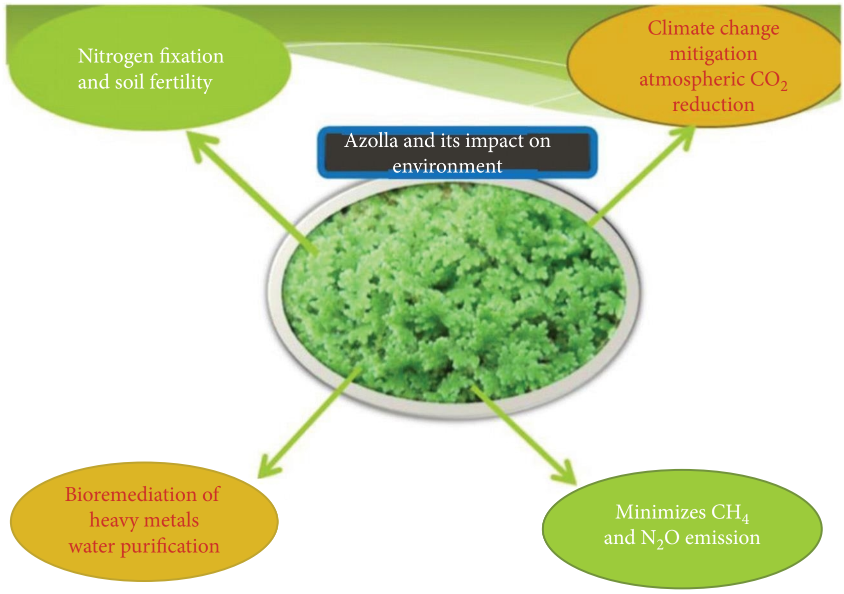

Figure 4: The importance of the Azolla plant in providing ecosystem services for sustainable aquaculture.

harmful pathogens, and enhancing the efficiency of nutrient absorption [130]. In conclusion, adding feed additives to Azolla feed for fish can be beneficial in terms of improving the nutrient profile and palatability. Fish can have a more balanced diet and better overall health by supplying probiotics, organic acid, and exogenous enzymes. Therefore, incorporating feed additives in Azolla feed is a practical and effective approach to sustainable aquaculture practices.

9. The Other Uses of the Azolla Plant and its Environmental Consequences

Azolla plant plays a vital role in the environment due to its nitrogen-fixing abilities, bioremediation properties, and carbon sequestration capabilities. Its ability to fix atmospheric nitrogen makes it an essential contributor to the fertility of aquatic ecosystems. This relationship has made Azolla and blue-green algae a popular choice for manure in rice cultivation, providing nitrogen to the soil and serving as feed for livestock and poultry. The integration of Azolla and rice has been shown to improve soil fertility and increase grain and straw production more effectively than using urea [131]. With the increasing pollution of the environment due to industrial, agricultural, and aquaculture waste, the use of Azolla as a feed ingredient is a promising solution [132]. Plants like Azolla contain less phosphate and nitrogen than animal protein feed, making them less likely to promote eutrophication in ponds [5]. Remediating accumulated harmful waste in water and soil is of utmost importance in today's world. With the rapid expansion of industries, agriculture, and urbanization, pollution is increasing on a daily basis. One of the major concerns is the emission, toxicity, and environmental stability of heavy metals, which have become significant sources of pollution [44]. Metal pollution directly affects the characteristics of water, leading to a decline in biological activity and a decrease in the availability of food in aquatic ecosystems. Consequently, when these pollutants enter the food chain, they pose a significant threat to the health of organisms [133]. The accumulation of heavy metals in fish tissues and aquatic ecosystems further exacerbates this hazard, as these metals eventually make their way into humans, birds, and other fish-eating animals [134, 135]. Research has shown that Azolla plants have the ability to remove hazardous chemicals from water through a process called bioremediation [136]. Moreover, Azolla plants play a crucial role in aquatic ecosystems' structural and functional aspects. They modify water movement, serve as habitats and food sources for fish and aquatic invertebrates, and influence water quality by regulating oxygen levels, nutrient cycles, and the accumulation of heavy metals [137]. In addition to its environmental benefits, Azolla meal has gained attention for its potential as an alternative feed for livestock due to its high protein content. However, concerns have been raised regarding the negative effects of heavy metal accumulation in Azolla when grown in contaminated water and used as feed for fish. Heavy metals such as lead, mercury, and cadmium are highly toxic to animals and can pose serious health risks if consumed in large quantities [138]. Therefore, extensive research is needed to investigate the impact of Azolla meal cultured in heavy metal-contaminated water on animal health before it can be considered a safe alternative feed.

Azolla could also convert ambient CO_2 and nitrogen (N_2) into carbohydrates ((CH_2O) n) and ammonia (NH_3), which it then decomposes to add nitrogen and organic carbon to the soil [139]. Using the Azolla plant as a biofertilizer, animal feed, medicine, water purifier, biogas generation, human food, mosquito control, and ammonia reduction contributes significantly to environmental preservation. When included in aquafeed, They can also be used as green manure for crops such as rice, wheat, water bamboo, taro, vegetables, bananas, and red spinach, resulting in 60%–80% of nitrogen mineralization [140, 141]. However, it is important to note that Azolla growth can be problematic in certain conditions where water has high nutrients. It may result in a thick, entire cover of the water's surface, leading to a loss of oxygen and a potential impact on aquatic animals. In addition, the decomposition of the plant may produce a powerful odor.

Despite these challenges, the benefits of Azolla plants are numerous. They can help eliminate methane from paddy fields, act as a mosquito repellent, and contribute significantly to environmental preservation [142]. As a mosquito repellent, Azolla has the potential to suppress mosquito populations. According to Rajendran and Reuben [143], the presence of Azolla can significantly reduce the likelihood of adult mosquitoes depositing eggs and larvae developing. *A. pinnata* can be utilized in biogas generation due to its rich composition of microelements such as lignin, cellulose, nitrogen, proteins, crude fibers, and solids. The presence of Azolla can have a significant impact on both mosquito populations and biogas production. Utilizing this natural resource can create a more sustainable future while reducing the spread of mosquito-borne diseases. Figure 4 below demonstrates the importance of the Azolla plant in providing ecosystem services for sustainable aquaculture.

10. Conclusion

This review delves into the impact of dietary Azolla meal components on fish, drawing from current literature publications. Azolla meal has been shown to contain high levels of crude protein, EAAs, vitamins, and minerals that support growth and development in aquatic species. According to literature, tilapia species can consume up to 20%, *Barbonymus gonionotus* up to 25%, common carps up to 15%, and *L. calbasu* up to 30%. Furthermore, it also has the potential to reduce the environmental impacts associated with conventional fishmeal production. Despite this, overall results indicate that using Azolla meal as a partial or complete replacement for fishmeal in aquafeed could benefit farmers by reducing feed costs. However, further research is needed to determine the optimal level of inclusion of Azolla meal in different species' diets and ensure its compatibility with other ingredients used in aquafeed manufacture.

11. Recommendations

Further research is required to address the following: feed containing a high percentage of Azolla plant ingredients requires special processing to remove the ANFs they contain. Although several processing methods claim to eliminate the ANFs found in plant feedstuffs, their effectiveness depends on the constituents. Standardizing the optimal processing techniques for all plant materials is crucial. Future studies should document the negative impacts of the Azolla meal use by fish, and appropriate technologies should be standardized to mitigate these effects. In addition, research should focus on the problems related to the increased use of Azolla meal by fish. The study should prioritize cheaper ingredients to evaluate Azolla ingredients based on their costs. When replacing fishmeals in fish diets, molecular tools should be used in feed nutrition studies. Researchers should investigate how genes involved in digestion, metabolism, and growth respond to the consumption of Azolla plant diets. These methods can identify the fish species that respond more favorably to Azolla plant diets than others. To create low-cost commercial feeds for those species, a fish feed may be prepared with a high percentage of plant-based ingredients. It is necessary to investigate the addition of feed additives such as probiotics and enzymes to Azolla meals to achieve the best feed utilization by boosting FCR, digestibility, disease protection, and immune system improvement. The Azolla plant is used as a biofertilizer, animal feed, and water purifier, contributing to environmental preservation. Therefore, research is constantly needed to increase Azolla production and aquaculture management for a better aquaculture industry.

Data Availability

Data would be available upon request.

Disclosure

This study did not receive any funding in any form but is part of Mpwaga Alatwinusa Yohana Ph.D. research project.

Conflicts of Interest

According to the authors, this review had no conflicts of interest.

Authors' Contributions

Mpwaga Alatwinusa Yohana and Gyan Watson Ray took part in writing the manuscript. Yang Qihui, Beiping Tan, Shuyan Chi, Amos Asase, Kou Shiyu, and Asare Derrick edited the manuscript. All the authors have approved the manuscript for the publication.

Acknowledgments

We want to thank our lab mates for their unwavering support. We appreciate the help from the Ministry of Agriculture's Key Laboratory of Aquatic, Livestock, and Poultry Feed Science and Technology in South China.

References

[1] S. M. Hixson, "Fish nutrition and current issues in aquaculture: the balance in providing safe and nutritious seafood, in an environmentally sustainable manner," *Journal of Aquaculture Research and Development*, vol. 5, no. 3, 2014.

[2] K. Hua, J. M. Cobcroft, A. Cole et al., "The future of aquatic protein: implications for protein sources in aquaculture diets," *One Earth*, vol. 1, no. 3, pp. 316–329, 2019.

[3] M. J. Sánchez-Muros, P. Renteria, A. Vizcaino, and F. G. Barroso, "Innovative protein sources in shrimp (*Litopenaeus vannamei*) feeding," *Reviews in Aquaculture*, vol. 12, no. 1, pp. 186–203, 2020.

[4] J. H. Cho and I. H. Kim, "Fish meal–nutritive value," *Journal of Animal Physiology and Animal Nutrition*, vol. 95, no. 6, pp. 685–692, 2011.

[5] M. S. Dorothy, S. Raman, V. Nautiyal, and K. Singh, "Use of potential plant leaves as ingredient in fish feed-A review," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 7, pp. 112–125, 2018.

[6] R. W. Hardy, "Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal," *Aquaculture Research*, vol. 41, no. 5, pp. 770–776, 2010.

[7] Y. Alfiko, D. Xie, R. T. Astuti, J. Wong, and L. Wang, "Insects as a feed ingredient for fish culture: status and trends," *Aquaculture and Fisheries*, vol. 7, no. 2, pp. 166–178, 2022.

[8] G. Savonitto, R. Barkan, S. Harpaz et al., "Fishmeal replacement by periphyton reduces the fish in fish out ratio and alimentation cost in gilthead sea bream *Sparus aurata*," *Scientific Reports*, vol. 11, no. 1, pp. 1–10, 2021.

[9] J. Zhang, Y. Dong, K. Song et al., "Effects of the replacement of dietary fish meal with defatted yellow mealworm (*Tenebrio molitor*) on juvenile large yellow croakers (*Larimichthys crocea*) growth and gut health," *Animals*, vol. 12, no. 19, Article ID 2659, 2022.

[10] S. Egerton, A. Wan, K. Murphy et al., "Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate," *Scientific Reports*, vol. 10, no. 1, pp. 1–16, 2020.

[11] S. Langyan, P. Yadava, F. N. Khan, Z. A. Dar, R. Singh, and A. Kumar, "Sustaining protein nutrition through plant-based foods," *Frontiers in Nutrition*, vol. 8, 2022.

[12] G. W. Ray, X. Li, S. He et al., "A review on the use of distillers dried grains with solubles (DDGS) in aquaculture feeds," *Annals of Animal Science*, vol. 22, no. 1, pp. 21–42, 2022.

[13] A. M. Lusiastuti and U. Paris-Saclay, "Can *Azolla filiculoides* be a complementary feed resource for ecological intensification in small-scale fish farming? Biological effects on giant gourami (*Osphronemus goramy*)," *Aquatic Living Resources*, vol. 36, Article ID 9, 2023.

[14] D. B. Mishra, D. Roy, V. Kumar et al., "Effect of feeding different levels of *Azolla pinnata* on blood biochemicals, hematology and immunocompetence traits of Chabro chicken," *Veterinary World*, vol. 9, no. 2, pp. 192–198, 2016.

[15] R. Nofiani, M. Kalbar, and P. Ardinisngsih, "Fisheries and aquatic science," *Journal of Fisheries and Aquatic Science*, vol. 8, no. 1, pp. 80–86, 2006.

[16] G. Agbagba, S. Asuming-Brempong, and I. Y. D. Lawson, "Dried *Azolla pinnata* as a supplementary nitrogen source for lowland rice production in a Calcic Natraquert," *Journal of Soil Science and Environmental Management*, vol. 9, no. 3, pp. 35–39, 2018.

[17] N. Adzman, S. J. Goh, A. Johari, M. N. H. Zainal Alam, and M. J. Kamaruddin, "Preliminary study on Azolla cultivation and characterization for sustainable biomass source," *Journal of Physics: Conference Series*, vol. 2259, no. 1, Article ID 012018, 2022.

[18] M. Akhtar, N. Sarwar, A. Ashraf, A. Ejaz, S. Ali, and M. Rizwan, "Beneficial role of *Azolla* sp. in paddy soils and their use as bioremediators in polluted aqueous environments: implications and future perspectives," *Archives of Agronomy and Soil Science*, vol. 67, no. 9, pp. 1242–1255, 2021.

[19] R. K. Yadav, K. Tripathi, P. W. Ramteke, E. Varghese, and G. Abraham, "Salinity induced physiological and biochemical changes in the freshly separated cyanobionts of *Azolla microphylla* and *Azolla caroliniana*," *Plant Physiology and Biochemistry*, vol. 106, pp. 39–45, 2016.

[20] J. Syamsiyah, B. H. Sunarminto, and M. Mujiyo, "Changes in soil chemical properties of organic paddy field with *Azolla* application," *Sains Tanah—Journal of Soil Science and Agroclimatology*, vol. 13, no. 2, Article ID 68, 2017.

[21] M. El-Deeb, H. Fahim, S. Shazly, M. Ragab, A. Alazab, and M. Beshara, "Effect of partially substitution of soybean protein with Azolla (*Azolla pinnata*) on productive performance and carcass traits of growing rabbits," *Journal of Animal and Poultry Production*, vol. 12, no. 6, pp. 197–203, 2021.

[22] B. Swain, P. Naik, S. Sahoo, S. Mishra, and D. Kumar, "Effect of feeding of Azolla (*Azolla pinnata*) on the performance of white pekin laying ducks," *International Journal of Livestock Research*, vol. 8, no. 5, Article ID 248, 2018.

[23] B. K. Swain, P. K. Naik, and C. K. Beura, "Nutritive value of *Azolla* as poultry feed-a review," *Indian Journal of Animal Nutrition*, vol. 39, no. 1, pp. 1–11, 2022.

[24] T. Ismail, E. Hegazi, E. Nassef, O. A. Habotta, and M. S. Gewaily, "The optimized inclusion level of *Bacillus subtilis* fermented *Azolla pinnata* in Nile tilapia (*Oreochromis niloticus*) diets: immunity, antioxidative status, intestinal digestive enzymes and histomorphometry, and disease resistance," *Fish Physiology and Biochemistry*, vol. 48, no. 3, pp. 767–783, 2022.

[25] F. I. Magouz, M. A. O. Dawood, M. F. I. Salem, and A. A. I. Mohamed, "The effects of fish feed supplemented with *Azolla* meal on the growth performance, digestive enzyme activity, and health condition of genetically-improved farmed tilapia (*Oreochromis niloticus*)," *Annals of Animal Science*, vol. 20, no. 3, pp. 1029–1045, 2020.

[26] S. N. Datta, "Culture of *Azolla* and its efficacy in diet of *Labeo rohita*," *Aquaculture*, vol. 310, no. 3-4, pp. 376–379, 2011.

[27] A. Goda, A. Saad, M. Hanafy, Z. Sharawy, and E. El-Haroun, "Dietary effects of *Azolla pinnata* combined with exogenous digestive enzyme (Digestin^{TM}) on growth and nutrients utilization of freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879)," *Journal of Oceanology and Limnology*, vol. 36, no. 4, pp. 1434–1441, 2018.

[28] S. Bara Scholar, S. Mishra, V. Khune, S. Bara, and S. Banjara, "Nutritional evaluation of *Azolla pinnata*," *The Pharma Innovation Journal*, vol. 9, no. 6, pp. 16-17, 2020.

[29] E. Coudert, E. Baéza, and C. Berri, "Use of algae in poultry production: a review," *World's Poultry Science Journal*, vol. 76, no. 4, pp. 767–786, 2020.

[30] K. A. Kabir, M. C. J. Verdegem, J. A. J. Verreth, M. J. Phillips, and J. W. Schrama, "Effect of dietary carbohydrate to lipid ratio on performance of Nile tilapia and enhancement of natural food in pond aquaculture," *Aquaculture Research*, vol. 51, no. 5, pp. 1942–1954, 2020.

[31] S. S. Mosha, S. Felix, and D. Manikandavelu, "Partial fishmeal replacement by *Azolla* meal on GIFT tilapia (*Oreochromis niloticus*) diet: effect on growth performance, antioxidant enzymes, immunology and stress response," *Acta Scientific Veterinary Sciences*, vol. 2, no. 8, pp. 17–25, 2020.

[32] P. Brouwer, H. Schluepmann, K. G. J. Nierop et al., "Growing *Azolla* to produce sustainable protein feed: the effect of differing species and CO_2 concentrations on biomass productivity and chemical composition," *Journal of the Science of Food and Agriculture*, vol. 98, no. 12, pp. 4759–4768, 2018.

[33] I. Ahmed, "Partial replacement of fishmeal by *Azolla cristata* in diets for fingerling common carp, *Cyprinus carpio* var. *communis*," pp. 1–15, 2022.

[34] S. B. Katole, S. R. Lende, and S. S. Patil, "A review on potential livestock feed: *Azolla*," *Livestock Research International*, vol. 5, no. 1, pp. 1–9, 2017.

[35] B. Chichilichi, G. P. Mohanty, S. K. Mishra et al., "Effect of partial supplementation of sun-dried *Azolla* as a protein source on the immunity and antioxidant status of commercial broilers," *Veterinary World*, vol. 8, no. 9, pp. 1126–1130, 2015.

[36] S. S. Mosha, "A review on significance of *Azolla* meal as a protein plant source in finfish culture," *Journal of Aquaculture Research & Development*, vol. 9, no. 7, 2018.

[37] C. C. C. R. de Carvalho and M. J. Caramujo, "Carotenoids in aquatic ecosystems and aquaculture: a colorful business with implications for human health," *Frontiers in Marine Science*, vol. 4, Article ID 93, 2017.

[38] A. Lejeune, J. Peng, E. Le Boulengé, Y. Larondelle, and C. Van Hove, "Carotene content of *Azolla* and its variations during drying and storage treatments," *Animal Feed Science and Technology*, vol. 84, no. 3-4, pp. 295–301, 2000.

[39] S. H. Marzouk, H. J. Tindwa, N. A. Amuri, and J. M. Semoka, "An overview of underutilized benefits derived from *Azolla* as a promising biofertilizer in lowland rice production," *Heliyon*, vol. 9, no. 1, Article ID e13040, 2023.

[40] O. A. Alalade and E. A. Iyayi, "Chemical composition and the feeding value of Azolla (*Azolla pinnata*) meal for egg-type chicks," *International Journal of Poultry Science*, vol. 5, no. 2, pp. 137–141, 2006.

[41] I. Khursheed, S. Masud, A. Khan et al., "Proximate evaluation of *Azolla pinnata* as sustainable feed supplement for poultry," *Journal of Pharmacognosy and Phytochemistry*, vol. 8, no. 3, pp. 3157–3160, 2019.

[42] I. Watanabe and N. S. Berja, "The growth of four species of *Azolla* as affected by temperature," *Aquatic Botany*, vol. 15, no. 2, pp. 175–185, 1983.

[43] E. Sjödin, "The Azolla Cooking," in *The Azolla Cooking and Cultivation Project*, Erik Sjödin, 2012.

[44] N. Cohen-Shoel, Z. Barkay, D. Ilzycer, I. Gilath, and E. Tel-Or, "Biofiltration of toxic elementary by Azolla biomass," *Water, Air, and Soil Pollution*, vol. 135, no. 1–4, pp. 93–104, 2002.

[45] T. T. Abdalbakee and H. N. Mohammed, "Effect of using different levels of Azolla as a substitute for soybean meal in the production performance of fish carp," *Plant Archives*, vol. 19, no. 1, pp. 573–577, 2019.

[46] D. Cooper, L. Doucet, and M. Pratt, "Understanding in multinational organizations," *Journal of Organizational Behavior*, vol. 28, no. 3, pp. 303–325, 2007.

[47] C. Kathirvelan, S. Banupriya, and M. R. Purushothaman, "Azolla-an alternate and sustainable feed for livestock," *International Journal of Science, Environment and Technology*, vol. 4, no. 4, pp. 1153–1157, 2015.

[48] K. Bhuvaneshwari and P. K. Singh, "Response of nitrogen-fixing water fern *Azolla* biofertilization to rice crop," *3 Biotech*, vol. 5, no. 4, pp. 523–529, 2015.

[49] P. Brouwer, A. Bräutigam, C. Külahoglu et al., "*Azolla* domestication towards a biobased economy?" *New Phytologist*, vol. 202, no. 3, pp. 1069–1082, 2014.

[50] D. P. Sarah Koti Ratnam, "Comparative studies on nutritive analysis and cultivation of four Azolla species," *Journal of Bio Innovation*, vol. 10, no. 1, pp. 147–156, 2021.

[51] G. N. Mathur, R. Sharma, and P. C. Choudhary, "Use of Azolla (*Azolla pinnata*) as cattle feed supplement," *Journal of Krishi Vigyan*, vol. 2, no. 1, pp. 73–75, 2013.

[52] T. A. Lumpkis, "Environmental requirements for successful Azolla growth," in *Azolla Utilization: Proceedings of the Workshop on Azolla Use Held in Fuzhou*, pp. 89–97, Fujian, China, March 31 to April 5, 1985, 1987, http://books.irri.org/9711041790_content.pdf.

[53] M. Das, F. Rahim, and M. Hossain, "Evaluation of fresh *Azolla pinnata* as a low-cost supplemental feed for thai silver barb *Barbonymus gonionotus*," *Fishes*, vol. 3, no. 1, pp. 1–11, 2018.

[54] A. Golzary, A. Hosseini, and M. Saber, "*Azolla filiculoides* as a feedstock for biofuel production: cultivation condition optimization," *International Journal of Energy and Water Resources*, vol. 5, no. 1, pp. 85–94, 2021.

[55] S. M. M. Shanab, "Effect of different percentages of Azolla cover on water quality, phytoplankton and fish production in fish ponds," in *Journal of the Union Arab Biologist, Botany (Physiology and Algae)*, vol. 8, pp. 1–19, 2001.

[56] G. L. Openjuru, "Makerere University Makerere University," 540009, 2019.

[57] N. Sanginga and C. Van Hove, "Amino acid composition of Azolla as affected by strains and population density," *Plant and Soil*, vol. 117, no. 2, pp. 263–267, 1989.

[58] R. J. M. Temmink, S. F. Harpenslager, A. J. P. Smolders et al., "Azolla along a phosphorus gradient: biphasic growth response linked to diazotroph traits and phosphorus-induced iron chlorosis," *Scientific Reports*, vol. 8, no. 1, pp. 3–10, 2018.

[59] M. Kumar, B. Patil, and Yamanura, "*Azoll*a the wonder fern," *Krishi Science*, vol. 1, Article ID 8, 2020.

[60] D. M. Cherryl, R. M. V. Prasad, and P. Jayalaxmi, "A study on economics of inclusion of Azolla pinnata in swine rations," *International Journal of Agricultural Sciences and Veterinary Medicine*, vol. 1, no. 4, pp. 50–56, 2013.

[61] M. A. Islam, "Effect of Azolla (Azolla pinnata) on growth and lipid profiles of broiler chickens," *Annals of Bangladesh Agriculture*, vol. 21, no. 1, pp. 73–78, 2017.

[62] G. Kumar and H. Chander, "A study on the potential of *Azolla pinnata* as livestock feed supplement for climate change adaptation and mitigation," *Asian Journal of Advanced Basic Sciences*, vol. 5, no. 2, pp. 65–68, 2017.

[63] Y.-L. Qiu and J. Yu, "Azolla—a model organism for plant genomic studies," *Genomics, Proteomics & Bioinformatics*, vol. 1, no. 1, pp. 15–25, 2003.

[64] M. E. J. da Silva, L. O. J. Mathe, I. L. van Rooyen, H. G. Brink, and W. Nicol, "Optimal growth conditions for *Azolla pinnata* R. brown: impacts of light intensity, nitrogen addition, pH control, and humidity," *Plants*, vol. 11, no. 8, Article ID 1048, 2022.

[65] A. Noviyanti, F. Husna, M. Ridhwan, and E. Surya, "The effectiveness of feeding *Azolla microphylla* on catfish (*Clarias batrachus*) growth," *Proceedings of International Conference on Multidiciplinary Research*, vol. 3, no. 1, pp. 58–70, 2020.

[66] B. Gangadhar, N. Sridhar, S. Saurabh et al., "Effect of Azolla-incorporated diets on the growth and survival of *Labeo fimbriatus* during fry-to-fingerling rearing," *Cogent Food and Agriculture*, vol. 1, no. 1, Article ID 1055539, 2015.

[67] H. Umalatha, B. Gangadhar, G. Hegde, and N. Sridhar, "Digestibility of three feed ingredients by *Catla catla* (Hamilton, 1822)," *Oceanography & Fisheries Open Access Journal*, vol. 5, no. 5, pp. 1–6, 2018.

[68] A.-F. M. El-Sayed, "Effect of substituting fish meal with *Azolla pinnata* in practical diets for fingerling and adult Nile tilapia, *Oreochromis niloticus* (L.)," *Aquaculture Research*, vol. 23, no. 2, pp. 167–173, 1992.

[69] N. Shiomi and S. Kitoh, "Culture of *Azolla* in a pond, nutrient composition, and use as fish feed," *Soil Science and Plant Nutrition*, vol. 47, no. 1, pp. 27–34, 2001.

[70] A.-F. M. El-Sayed and D. L. Garling, "Carbohydrate-to-lipid ratios in diets for *Tilapia zillii* fingerlings," *Aquaculture*, vol. 73, no. 1–4, pp. 157–163, 1988.

[71] B. Columbia, J. Maity, B. C. Patra, W. Bengal, and W. Bengal, "Effect of replacement of fishmeal by *Azolla* leaf mael on growth, food utilization, pancreatic protease activity and RNA/DNA ratio in the fingerlings of *Labeo rohita* (Ham.)," *Canadian Journal of Pure and Applied Sciences*, vol. 2, no. 2, pp. 323–333, 2008.

[72] I. Ahmed, Y. M. Khan, A. Lateef, K. Jan, A. Majeed, and M. A. Shah, "Effect of fish meal replacement by *Azolla* meal on growth performance, hemato-biochemical and serum parameters in the diet of scale carp, *Cyprinus carpio* var. *communis*," *Journal of the World Aquaculture Society*, vol. 54, no. 5, pp. 1301–1316, 2023.

[73] A. Youssouf, "Water quality and sediment features in ponds with Nile tilapia (*Oreochromis niloticus*) fed *Azolla*," *Journal of Fisheries and Aquaculture*, vol. 3, no. 2, pp. 47–51, 2012.

[74] J. Wang, Y. Jiang, X. Li et al., "Dietary protein requirement of juvenile red spotted grouper (*Epinephelus akaara*)," *Aquaculture*, vol. 450, pp. 289–294, 2016.

[75] S. Rahmah, U. Nasrah, L. S. Lim, S. D. Ishak, M. Z. H. Rozaini, and H. J. Liew, "Aquaculture wastewater-raised *Azolla* as partial alternative dietary protein for Pangasius catfish," *Environmental Research*, vol. 208, Article ID 112718, 2022.

[76] M. M. Refaey, A. I. Mehrim, O. A. Zenhom, H. A. Areda, J. A. Ragaza, and M. S. Hassaan, "Fresh Azolla, *Azolla pinnata* as a complementary feed for *Oreochromis niloticus*: growth, digestive enzymes, intestinal morphology, physiological responses, and flesh quality," *Aquaculture Nutrition*, vol. 2023, Article ID 1403704, 13 pages, 2023.

[77] C. O. Mendivil, "Dietary fish, fish nutrients, and immune function: a review," *Frontiers in Nutrition*, vol. 7, pp. 1–9, 2021.

[78] S. A. M. Martin and E. Król, "Nutrigenomics and immune function in fish: new insights from omics technologies," *Developmental and Comparative Immunology*, vol. 75, pp. 86–98, 2017.

[79] M. A. O. Dawood, "Nutritional immunity of fish intestines: important insights for sustainable aquaculture," *Reviews in Aquaculture*, vol. 13, no. 1, pp. 642–663, 2021.

[80] K. Mzengereza, M. Ishikawa, S. Koshio et al., "novo camelina meal in diets of red seabream (*Pagrus major*)," 2021.

[81] W. B. van Muiswinkel and B. Vervoorn-Van der Wal, "The immune system of fish," *Fish Diseases and Disorders*, vol. 1, pp. 678–701, 2006.

[82] H. Schulenburg, C. L. Kurz, and J. J. Ewbank, "Evolution of the innate immune system: the worm perspective," *Immunological Reviews*, vol. 198, no. 1, pp. 36–58, 2004.

[83] A. E. Ellis, "Innate host defense mechanisms of fish against viruses and bacteria," *Developmental and Comparative Immunology*, vol. 25, no. 8-9, pp. 827–839, 2001.

[84] C. Uribe, H. Folch, R. Enriquez, and G. Moran, "Innate and adaptive immunity in teleost fish: a review," *Veterinarni Medicina*, vol. 56, no. 10, pp. 486–503, 2011.

[85] J. Holmgren and C. Czerkinsky, "Mucosal immunity and vaccines," *Nature Medicine*, vol. 11, no. S4, pp. S45–S53, 2005.

[86] J. W. Bledsoe, M. R. Pietrak, G. S. Burr, B. C. Peterson, and B. C. Small, "Functional feeds marginally alter immune expression and microbiota of Atlantic salmon (*Salmo salar*) gut, gill, and skin mucosa though evidence of tissue-specific signatures and host–microbe coadaptation remain," *Animal Microbiome*, vol. 4, no. 1, Article ID 20, 2022.

[87] C. Lumsangkul, N. Vu Linh, F. Chaiwan et al., "Dietary treatment of Nile tilapia (*Oreochromis niloticus*) with aquatic fern (*Azolla caroliniana*) improves growth performance, immunological response, and disease resistance against *Streptococcus agalactiae* cultured in bio-floc system," *Aquaculture Reports*, vol. 24, Article ID 101114, 2022.

[88] T. L. N. Tran, A. F. Miranda, S. W. Abeynayake, and A. Mouradov, "Differential production of phenolics, lipids, carbohydrates and proteins in stressed and unstressed aquatic plants, *Azolla filiculoides* and *Azolla pinnata*," *Biology*, vol. 9, no. 10, pp. 1–15, 2020.

[89] A. Alesci, S. Pergolizzi, S. Savoca et al., "Detecting intestinal goblet cells of the broadgilled hagfish *Eptatretus cirrhatus* (Forster, 1801): a confocal microscopy evaluation," *Biology*, vol. 11, no. 9, pp. 1–11, 2022.

[90] H. Bjørgen and E. O. Koppang, "Anatomy of teleost fish immune structures and organs," *Immunogenetics*, vol. 73, no. 1, pp. 53–63, 2021.

[91] B. Magnadóttir, "Innate immunity of fish (overview)," *Fish and Shellfish Immunology*, vol. 20, no. 2, pp. 137–151, 2006.

[92] S. Dash, S. K. Das, J. Samal, and H. N. Thatoi, "Epidermal mucus, a major determinant in fish health: a review," *Iranian Journal of Veterinary Research*, vol. 19, no. 2, pp. 72–81, 2018.

[93] P. Ferraboschi, S. Ciceri, and P. Grisenti, "Applications of lysozyme, an innate immune defense factor, as an alternative antibiotic," *Antibiotics*, vol. 10, no. 12, pp. 1–55, 2021.

[94] J. D. Biller, G. D. V. Polycarpo, B. S. Moromizato et al., "Lysozyme activity as an indicator of innate immunity of tilapia (*Oreochromis niloticus*) when challenged with LPS and *Streptococcus agalactiae*," *Revista Brasileira de Zootecnia*, vol. 50, pp. 1–10, 2021.

[95] L. Wu, Z. Qin, H. Liu, L. Lin, J. Ye, and J. Li, "Recent advances on phagocytic B cells in teleost fish," *Frontiers in Immunology*, vol. 11, pp. 1–9, 2020.

[96] E. Hegazi, E. Nassef, O. A. Habotta, and M. S. Gewaily, "The optimized inclusion level of Bacillus subtilis fermented Azolla pinnata in Nile tilapia (Oreochromis niloticus) diets: immunity, antioxidative status, intestinal digestive enzymes and histomorphometry, and disease resistance," *Fish Physiology and Biochemistry*, vol. 48, no. 3, pp. 767–783, 2022.

[97] S. S. Mosha, S. Felix, D. Manikandavelu, N. Felix, T. S. Moses, and M. Menaga, "Effect of dietary mixture containing *Azolla* and spirulina platensis on physiological, metabolic, immunological and histological performance of GIFT tilapia (*Oreochromis niloticus*) cultured in lined ponds," *Advances in Oceanography & Marine Biology*, vol. 2, no. 1, pp. 1–11, 2020.

[98] A. R. Deshmukh and D. A. Nalle, "Evaluation of improvement in immunity after application of dietary *Azolla pinnata*, a study of blood serum parameters of *Cyprinus carpio* fingerlings after challenge of *Aeromonas hydrophila*," *Xi'an Shiyou Xueyuan Xuebao/Journal of Xi'an Petroleum Institute (Natural Science Edition)*, vol. 18, 2022.

[99] I. Ahmed and I. Ahmad, "Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, *Oncorhynchus*

mykiss reared in Indian Himalayan region," *Aquaculture Reports*, vol. 16, Article ID 100268, 2020.

[100] S. M. Shaima, R. H. Bakhan, F. N. Karzan, M. R. Rabar, A. S. Avan, and M. A. Nasreen, "Impact of natural *Azolla filiculoides* powders on some physiological, nutritional and biological parameters of common carp (*Cyprinus carpio* L.)," *Applied Ecology and Environmental Research*, vol. 18, no. 4, pp. 4959–4968, 2020.

[101] Q. Liang, M. Yuan, L. Xu et al., "Application of enzymes as a feed additive in aquaculture," *Marine Life Science and Technology*, vol. 4, no. 2, pp. 208–221, 2022.

[102] V. Kumar, S. Hossain, J. A. Ragaza, and M. R. Benito, "The potential impacts of soy protein on fish gut health," in *Soybean for Human Consumption and Animal Feed*, vol. 2020, IntechOpen, 2020.

[103] J. D. Biller-Takahashi and E. C. Urbinati, "Fish immunology. The modification and manipulation of the innate immune system: Brazilian studies," *Anais da Academia Brasileira de Ciências*, vol. 86, pp. 1483–1495, 2014.

[104] C. J. Secombes and T. C. Fletcher, "The role of phagocytes in the protective mechanisms of fish," *Annual Review of Fish Diseases*, vol. 2, no. C, pp. 53–71, 1992.

[105] M. Kumar, R. Dhuria, D. Jain, T. Sharma, R. Nehra, and L. Gupta, "Effect of supplementation of Azolla on the hematology, immunity and gastrointestinal profile of broilers," *International Journal of Livestock Research*, vol. 8, no. 5, Article ID 184, 2018.

[106] B. G. Harmon, "Avian heterophils in inflammation and disease resistance," *Poultry Science*, vol. 77, no. 7, pp. 972–977, 1998.

[107] K. Vikas, B. Debtanu, K. Kundan, K. Vikash, S. C. Mandal, and E. D. Clercq, "Anti-nutritional factors in plant feedstuffs used in aquafeeds," *World Aquaculture*, vol. 43, no. 3, pp. 64–68, 2012.

[108] M. T. Awulachew, "A review of anti-nutritional factors in plant based foods," *Food Science & Nutrition Research, Ethiopian Institute of Agricultural Research*, vol. 7, no. 3, pp. 223–236, 2022.

[109] R. M. Maas, M. C. J. Verdegem, S. Debnath, L. Marchal, and J. W. Schrama, "Effect of enzymes (phytase and xylanase), probiotics (*B. amyloliquefaciens*) and their combination on growth performance and nutrient utilisation in Nile tilapia," *Aquaculture*, vol. 533, Article ID 736226, 2021.

[110] C. J. Solis, M. K. Hamilton, M. Caruffo et al., "Intestinal inflammation induced by soybean meal ingestion increases intestinal permeability and neutrophil turnover independently of microbiota in zebrafish," *Frontiers in Immunology*, vol. 11, pp. 1–11, 2020.

[111] N. Bai, M. Gu, M. Liu, Q. Jia, S. Pan, and Z. Zhang, "Corn gluten meal induces enteritis and decreases intestinal immunity and antioxidant capacity in turbot (*Scophthalmus maximus*) at high supplementation levels," *PLoS One*, vol. 14, no. 3, Article ID e0213867, 2019.

[112] Å. Krogdahl, K. Gajardo, T. M. Kortner et al., "Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.)," *Journal of Agricultural and Food Chemistry*, vol. 63, no. 15, pp. 3887–3902, 2015.

[113] M. Samtiya, R. E. Aluko, and T. Dhewa, "Plant food anti-nutritional factors and their reduction strategies: an overview," *Food Production, Processing and Nutrition*, vol. 2, no. 1, pp. 1–14, 2020.

[114] A. Duraiswamy, N. M. Sneha A., S. Jebakani K. et al., "Genetic manipulation of anti-nutritional factors in major crops for a sustainable diet in future," *Frontiers in Plant Science*, vol. 13, pp. 1–26, 2023.

[115] B. H. Vagadia, S. K. Vanga, and V. Raghavan, "Inactivation methods of soybean trypsin inhibitor—a review," *Trends in Food Science and Technology*, vol. 64, pp. 115–125, 2017.

[116] M. H. M. Yacout, "Anti-nutritional factors & its roles in animal nutrition," *Journal of Dairy, Veterinary & Animal Research*, vol. 4, no. 1, pp. 239–241, 2016.

[117] I. G. Borlongan, R. M. Coloso, and N. V. Golez, "Feeding habits and digestive physiology of fishes," in *Nutrition in Tropical Aquaculture: Essentials of Fish Nutrition, Feeds, and Feeding of Tropical Aquatic Species*, O. M. Millamena, R. M. Coloso, and F. P. Pascual, Eds., pp. 77–97, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines, 2002.

[118] K. W. Buckingham, S. W. Ela, J. G. Morris, and C. R. Goldman, "Nutritive value of the nitrogen-fixing aquatic fern *Azolla filiculoides*," *Journal of Agricultural and Food Chemistry*, vol. 26, no. 5, pp. 1230–1234, 1978.

[119] E. A. Fasakin, A. M. Balogun, and O. A. Fagbenro, "Evaluation of sun-dried water fern, *Azolla africana* and Duckweed, *Spirodela polyrrhiza* in practical diets for Nile tilapia, *Oreochromis niloticus* fingerlings," *Journal of Applied Aquaculture*, vol. 11, no. 4, pp. 83–92, 2001.

[120] E. D. Fiogbé, J.-C. Micha, and C. Van Hove, "Use of a natural aquatic fern, *Azolla microphylla*, as a main component in food for the omnivorous-phytoplanktonophagous tilapia, *Oreochromis niloticus* L." *Journal of Applied Ichthyology*, vol. 20, no. 6, pp. 517–520, 2004.

[121] A. Sh, K. Munish, G. Gyandeep, P. Neeraj, and M. Varun, "A review on replacing fish meal in aqua feeds using plant and animal protein sources," *International Journal of Chemical Studies*, vol. 7, no. 3, pp. 4732–4739, 2019.

[122] I. Oktavianawati, D. Andinata, A. N. Isnaeni et al., "Effects of feeding diets containing *Azolla pinnata* and probiotic on the growth and nutritional content of patin fish (*Pangasius djambal*)," *Agriculture and Agricultural Science Procedia*, vol. 9, pp. 403–410, 2016.

[123] W. J. Jang, J. M. Lee, M. T. Hasan, B.-J. Lee, S. G. Lim, and I.-S. Kong, "Effects of probiotic supplementation of a plant-based protein diet on intestinal microbial diversity, digestive enzyme activity, intestinal structure, and immunity in olive flounder (*Paralichthys olivaceus*)," *Fish and Shellfish Immunology*, vol. 92, pp. 719–727, 2019.

[124] O. Oluwatosin, "Effects of feed additives in fish feed for improvement of aquaculture," *Eurasian Journal of Food Science and Technology*, vol. 3, no. 2, pp. 49–57, 2019.

[125] S. Ganguly, K. C. Dora, S. Sarkar, and S. Chowdhury, "Supplementation of prebiotics in fish feed: a review," *Reviews in Fish Biology and Fisheries*, vol. 23, no. 2, pp. 195–199, 2013.

[126] T. N. Amer, M. E. A. Seden, and N. E. El-Tawil, "Effect of dietary phytase levels supplementation in improving soybean meal-based diets efficiency for Nile tilapia (*Oreochromis niloticus*)," *SVU-International Journal of Environmental Researches*, vol. 1, no. 2, pp. 69–80, 2019.

[127] X. Liang, X. Luo, H. Lin et al., "Growth, health, and gut microbiota of female pacific white shrimp, *Litopenaeus vannamei* broodstock fed different phospholipid sources," *Antioxidants*, vol. 11, no. 6, Article ID 1143, 2022.

[128] S. Castillo and D. M. Gatlin III, "Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review," *Aquaculture*, vol. 435, pp. 286–292, 2015.

[129] J. J. Dibner and P. Buttin, "Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism," *Journal of Applied Poultry Research*, vol. 11, no. 4, pp. 453–463, 2002.

[130] A. Bedford and J. Gong, "Implications of butyrate and its derivatives for gut health and animal production," *Animal Nutrition*, vol. 4, no. 2, pp. 151–159, 2018.

[131] D. P. Bartholomew, J. A. Silva, and D. J. C. Friend, "Study of the physiology of Azolla and its use," 1986.

[132] A. L. Singh and P. K. Singh, "Comparative studies on different methods of *Azolla* utilization in rice culture," *The Journal of Agricultural Science*, vol. 107, no. 2, pp. 273–278, 1986.

[133] A. M. Banach, A. Kuźniar, J. Grządziel, and A. Wolińska, "*Azolla filiculoides* L. as a source of metal-tolerant microorganisms," *PLoS One*, vol. 15, no. 5, Article ID e0232699, 2020.

[134] A. A. M. Abd El-All, E. M. Aref, and H. A. M. Hassanein, "Bioaccumulation of heavy metals by the water fern *Azolla pinnata*," *Egyptian Journal of Agricultural Research*, vol. 89, no. 4, pp. 1261–1276, 2011.

[135] M. Anand, B. Kumar, and R. Sheel, "Effect of heavy metals on biochemical profile of *Azolla filiculoides*," *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 10, pp. 3629–3653, 2017.

[136] A. Sood, P. L. Uniyal, R. Prasanna, and A. S. Ahluwalia, "Phytoremediation potential of aquatic macrophyte, *Azolla*," *AMBIO*, vol. 41, no. 2, pp. 122–137, 2012.

[137] M. K. Devi, W. N. Singh, W. R. C. Singh, H. B. Singh, and N. M. Singh, "Determination of the ability of Azolla as an agent of bioremediation," *European Journal of Experimental Biology*, vol. 4, no. 4, pp. 52–56, 2014.

[138] M. Hassanzadeh, R. Zarkami, and R. Sadeghi, "Uptake and accumulation of heavy metals by water body and *Azolla filiculoides* in the Anzali wetland," *Applied Water Science*, vol. 11, no. 6, pp. 1–8, 2021.

[139] S. Amit, K. Amit, P. Anoop, and K. Ashok, "Azolla-an environment eco-friendly pteridophytic species," *European Journal of Biomedical and Pharmaceutical Sciences*, vol. 3, no. 6, pp. 210–213, 2019.

[140] O. Ito and I. Watanabe, "Availability to rice plants of nitrogen fixed by *Azolla*," *Soil Science and Plant Nutrition*, vol. 31, no. 1, pp. 91–104, 1985.

[141] D. P. Widiastuti, J. G. Davis, and S. Gafur, "Azolla fertilizer as an alternative N source for red spinach production on alluvial and peat soils in West Kalimantan, Indonesia," in *International Nitrogen Initiative Conference*, November, 2–5, Melbourne, Australia, 2018

[142] D. Soman, V. Anitha, and A. Arora, "Bioremediation of municipal sewage water with *Azolla microphylla*," *International Journal of Advanced Research*, vol. 6, no. 5, pp. 101–108, 2018.

[143] R. Rajendran and R. Reuben, "Laboratory evaluation of the water fern, *Azolla pinnata* for mosquito control," *Journal of Biological Control*, vol. 2, pp. 114–116, 1988.