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DEVELOPING SUSTAINABLE FEED INGREDIENTS FOR FISH NUTRITION AND CLIMATE RESILIENT AQUACULTURE

EDITION: 2024

EDITED BY

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Developing Sustainable Feed Ingredients for Fish Nutrition and Climate Resilient Aquaculture

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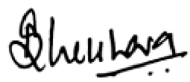
MESSAGE

National Institute of Agricultural Extension Management (MANAGE), Hyderabad is an autonomous organization under the Ministry of Agriculture & Farmers Welfare, Government of India. The policies of liberalization and globalization of the economy and the level of agricultural technology becoming more sophisticated and complex, called for major initiatives towards reorientation and modernization of the agricultural extension system. Effective ways of managing the extension system needed to be evolved and the extension organizations enabled to transform the existing set up through professional guidance and training of critical manpower. MANAGE is the response to this imperative need. Agricultural extension to be effective, demands sound technological knowledge to the extension functionaries and therefore MANAGE has focused on training program on technological aspect in collaboration with ICAR institutions and State Agriculture/Horticulture Universities, who has expertise and facilities to organize technical training program for extension functionaries of state/central fisheries departments, industrialists, and faculty of SAUs/KVKs/ICAR institutes.

Research and development efforts have been directed towards identifying alternative protein and nutrient sources that reduce dependency on wild fisheries and minimize the environmental impact of aquafeed production. This shift encompasses the exploration of plant-based proteins, single-cell proteins, and other novel ingredients that not only meet the nutritional requirements of aquatic species but also contribute to a more sustainable and circular approach within the industry. Furthermore, the emphasis on sustainable aquafeed ingredients aligns with broader goals of minimizing the ecological footprint of aquaculture operations. This includes addressing issues such as overfishing, reducing reliance on fishmeal and fish oil derived from marine sources, and exploring locally sourced and resilient feed components. The collaborative efforts of researchers, industry professionals, and policymakers have led to the formulation of eco-friendly aquafeeds and best practices for integrating sustainable feed ingredients. This holistic approach aims to balance the nutritional needs of aquatic species with the long-term health of aquatic ecosystems. As the aquaculture sector continues to evolve, the development of sustainable feed ingredients stands as a testament to the industry's commitment to responsible and forward-thinking practices. This online training program address the key areas of the sustainable feed ingredients and its implication in aquaculture.

It is a pleasure to note that, Aquaculture Technology & Research Foundation India (AQUAFIN), Chennai and MANAGE, Hyderabad is organizing a collaborative training program entitled “Developing Sustainable Feed Ingredients for Fish Nutrition and Climate Resilient Aquaculture” from 26-28 June, 2024 and coming up a joint publication as *e*- book as immediate outcome of the training program.

I wish the program be very purposeful and meaningful to the participants and also the *e*-book will be useful for stakeholders across the country. I extend my best wishes for success of the program and also I wish Aquaculture Technology & Research Foundation India (AQUAFIN) many more glorious years in service of Indian agriculture and allied sector ultimately benefitting the farmers. I would like compliment the efforts of Program Coordinators of MANAGE, Hyderabad and Aquaculture Technology & Research Foundation India (AQUAFIN) for this valuable publication.



(P. CHANDRA SHEKARA)

Director General, MANAGE

FOREWORD

Developing sustainable feed ingredients for climate-resilient aquaculture is not just an innovative strategy but an essential step to secure the future of global food security. As the world confronts the growing challenges posed by climate change, it is evident that the aquaculture industry must adapt by embracing practices that emphasize environmental sustainability and resilience. The reliance on traditional feed ingredients, such as those sourced from wild-caught fish or land-based crops, is proving to be unsustainable. These practices are contributing to the depletion of marine resources, the destruction of natural habitats, and the intensifying competition for agricultural resources—factors that collectively worsen the very climate challenges we seek to address.

To secure the future of aquaculture, there is an urgent need to develop alternative feed sources that significantly reduce the industry's ecological footprint. Sustainable feed ingredients, such as those derived from innovative sources like microalgae, insect proteins, and plant-based materials, present promising and practical solutions. These alternatives offer numerous benefits: they alleviate the pressure on overexploited wild fish stocks, reduce the demand for increasingly scarce agricultural land, and promote the principles of a circular economy by repurposing waste products and byproducts. Moreover, these new feed ingredients can be engineered to improve the resilience of farmed aquatic species to the adverse effects of climate change, including rising temperatures and deteriorating water quality. By fostering a strong commitment to sustainability and investing in cutting-edge research, we can ensure that the aquaculture industry not only meets the growing global demand for seafood but also contributes to the preservation of our planet's health.

This foreword highlights the urgent need for sustainable feed development within the broader context of climate change and global food security. It serves as a call to action for all stakeholders in the aquaculture sector to prioritize environmental stewardship and drive innovation, ensuring that our aquatic food systems remain viable and sustainable for generations to come.

I extend my heartfelt thanks to MANAGE (National Institute of Agricultural Extension and Management) for their invaluable contribution and continued support for this initiative. MANAGE has played a pivotal role in training researchers and students in this emerging technology. The involvement of several esteemed faculty members from central government institutes has greatly bolstered our confidence in advancing this technology to the next level. I also want to acknowledge the dedicated efforts of our team in successfully conducting this training program and express my gratitude to all the participants who all are attended our training program.

(S. Felix)

President, AQUAFIN

PREFACE

This *e*-book is an outcome of collaborative online training program on "Developing Sustainable Feed Ingredients for Fish Nutrition and Climate Resilient Aquaculture" from 26-28 June, 2024. The editors' main aim is to provide insights to all extension workers, faculties, researchers and students about developing sustainable feed ingredients for fish nutrition and climate resilient aquaculture.

This book explores the critical role of sustainable feed development in shaping the future of aquaculture amidst the growing challenges of climate change. As global demand for seafood continues to rise, the aquaculture industry faces increasing pressure to adopt practices that are both environmentally sustainable and resilient to climate variability. Traditional feed ingredients, often sourced from wild-caught fish or conventional agriculture, are becoming less viable due to overfishing, environmental degradation, and the competition for land-based resources. The book delves into innovative alternatives, such as microalgae, insect proteins, and plant-based feed ingredients, which offer promising solutions for reducing the industry's ecological footprint while enhancing the resilience of farmed species to changing environmental conditions. Additionally, the book provides insights into the collaborative efforts needed among researchers, industry leaders, and policymakers to accelerate the adoption of these sustainable practices. This e-book is an essential resource for researchers, industry professionals, policymakers, and students who are engaged in the fields of aquaculture, environmental sustainability, and food security. It offers a forward-looking perspective on how the aquaculture industry can evolve to meet the challenges of the future while contributing to the health of our planet.

The editors felt that all the experience of resource persons of this training should be integrated together to form a unique proposition on Sustainable Feed Ingredients for Fish Nutrition and Climate Resilient Aquaculture. The experts and resource persons in fisheries science contributed immensely and tirelessly to develop various chapters of this e-book in very short span of time. They all deserve applaud. The editors extend their sincere thanks to all the experts who have contributed valuable time and put sincere efforts to produce this *e*-book.

The editors also thank MANAGE, Hyderabad for the financial support to the training program. The editor's express gratitude towards the President, AQUAFIN for his constant encouragement and *e*-book publication to the participants. The editors hope that this *e*-book will help participants as well as other extension people across the country to gain valuable information on Developing Sustainable Feed Ingredients for Fish Nutrition and Climate Resilient Aquaculture.

Editors

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K Sai Maheswari

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Sustainable Feed Ingredients and Fish Health

Husain Nottanalan and Ashutosh D Deo

ICAR-CIFE Mumbai

1. Introduction

The sustainability of aquaculture practices has become a focal point of global environmental and food security discussions. Central to this dialogue is the role of sustainable feed ingredients in maintaining the health and well-being of farmed fish (Van Doan *et al.,* 2021). Traditionally, the aquaculture industry has heavily relied on fishmeal and fish oil sourced from wild-caught fish, a practice that has raised significant concerns regarding overfishing, habitat destruction, and the depletion of marine resources (Fawole *et al.,* 2023). As such, there is an urgent need to transition towards more sustainable and innovative feed alternatives that can mitigate these environmental impacts while promoting fish health. Sustainable feed ingredients not only enhance the environmental and social sustainability of aquaculture but also play a critical role in supporting the physiological health of fish (Galkanda-Arachchige *et al.,* 2020). By carefully assessing the nutritional value, environmental impact, and health implications of feed ingredients, aquaculture can adopt a holistic approach that supports fish growth and development while minimizing adverse effects on aquatic ecosystems (Turchini *et al.,* 2019). The quality and safety of feed ingredients are paramount, as they directly influence the overall health, resilience, productivity, and disease resistance of farmed fish (Kari *et al.*, 2022). Exploring alternative and innovative feed ingredients is essential to reduce reliance on marine-derived resources, minimize waste, and foster a more circular approach to feed production. A deep understanding of the nutritional requirements of various fish species, coupled with the potential benefits of specific feed components, can lead to the development of optimized diets tailored to enhance growth, immune function, and overall health (Hamid *et al.,* 2022). Prioritizing sustainable feed ingredients is thus imperative for advancing towards an environmentally responsible and resilient aquaculture industry, benefiting both farmed fish and the ecosystems in which they are cultivated (Dawood and Koshio, 2020).

2. Challenges in Using and Replacing Fish Meal and Fish Oil

The challenges in using and replacing fish meal and fish oil in aquaculture are complex and crucial for sustainability. Marine shrimp, fishes, and anadromous salmon are major consumers of fish meal, meeting their high protein needs for metabolic functions (Tacon *et al.,* 2011; Tacon and Metian, 2015). Similarly, anadromous salmon, marine fishes, and trout heavily rely on fish oil for essential polyunsaturated fatty acids like EPA and DHA (Torstensen *et al.,* 2005). However, inclusion rates of fish oil vary widely among species, with herbivorous and omnivorous freshwater fishes needing less compared to carnivorous species (FAO, 2021). Substitutes, mainly plant-based, often have lower digestibility and less favourable amino acid profiles, along with antinutritional factors that can affect growth (Gatlin *et al.,* 2007). Despite research into alternatives such as insects offering nutritional benefits, challenges persist in terms of costs, processing, and ensuring consistent quality (Makkar *et al.,* 2014). Addressing these issues is crucial for sustainable aquaculture, reducing environmental impact, and improving resource efficiency.

3. Advancing Sustainable Aquaculture through Agricultural Waste and Animal by product

Transitioning to sustainable aquaculture emphasizes shifting to alternative feed ingredients from agricultural waste. Traditional reliance on fishmeal and fish oil raises environmental concerns, urging exploration of sustainable options (Agrawal *et al.,* 2018). By using by-products like rice bran, soy pulp, and palm oil residues, and other animal waste for aquafeed, aquaculture reduces its ecological footprint while boosting fish health. Addressing challenges such as anti-nutritional factors and pesticide residues requires innovative solutions. Integrating agricultural waste into aquafeed is key to achieving sustainable aquaculture, benefiting both industry and the environment (Webber *et al.,* 2014).

4. Sustainability of Using Agricultural Waste in Aquaculture

4.1. Crop agricultural waste

4.1.1. Rice bran

India's projected paddy rice production of 127 million metric tons in 2023-24 has significant implications for the global output of rice bran, a valuable by-product comprising 8-10% of the rice grain. This nutrient-rich bran layer, removed during rice milling, represents a substantial source of animal feed and aquaculture inputs. Global rice bran production reaches 63 million tonnes annually, primarily utilized as animal feed due to its rich nutritional profile. Rice bran demonstrates considerable value in aquaculture, serving as both feed and fertilizer. Studies highlight its efficacy as a sole feed for tilapia and its potential in fermented form to enhance shrimp production and boost tilapia growth. Further research underscores the benefits of fermented rice bran in biofloc technology for catfish growth, white leg shrimp cultivation, and as a probiotic promoter in jelawat farming. The proximate composition of Rice Bran is shown in the table 1. In addition to the proximate composition, rice bran also contains various beneficial compounds. Phenolic compounds, including free phenolics, bound phenolics, and total phenolics, are present. Mardiah et al., (2024) found that defatted rice bran retained 88% of the phenolics compared to non-defatted rice bran. Rice bran is also a good source of B vitamins, vitamin E, and minerals such as magnesium, phosphorus, and manganese. Furthermore, it is an excellent source of dietary fiber, which is beneficial for digestive health. Additionally, rice bran contains various antioxidants, including tocopherols, tocotrienols, and oryzanol. The defatting process primarily affects the lipid content, as would be expected. However, it can also impact the levels of other components, such as phenolics.

Component	Non-defatted Rice Bran	Defatted Rice Bran
Moisture (%)	2.3 ± 0.03	5.5 ± 0.01
Protein (%)	15.4 ± 0.10	17.8 ± 0.09
Lipid (%)	15.3 ± 1.3	0.6 ± 0.03
Ash (%)	7.9 ± 0.06	9.0 ± 0.06
NFE (%)	37.1 ± 0.02	39.34 ± 0.05
Fibre (%)	7.45 ± 0.03	13.65 ± 0.0

Table1: proximate composition of Rice bran (Ranjan *et al.,* 2023)

4.1.2. Wheat bran Wheat bran, the outer layer of the wheat kernel, is a nutrient-dense byproduct of wheat milling. Its proximate composition reveals a rich source of dietary fiber, primarily composed of arabinoxylans and cellulose. These fibers contribute to satiety, promote gut health. Additionally, wheat bran provides significant amounts of protein, vitamins (including B vitamins and vitamin E), and minerals like magnesium, phosphorus, and manganese. Furthermore, it contains bioactive compounds like phenolic acids and lignans, which exhibit antioxidant and anti-inflammatory properties. While wheat bran's coarse texture can impact palatability, its incorporation into food products offers numerous health benefits and aligns with dietary recommendations for increased fiber intake.Wheat bran, a fiber-rich by-product of wheat flour milling, contains 16% protein, 5% lipid, 6% ash, 12% carbohydrate, and 43% fiber. Due to its high fiber content, it is rarely used in fish feed. Tables 2 present the data on wheat production.

Country/region	2022/23 (MMT)	2023/24 May (MMT)
World total	788.3	789.8
China	137.7	140.0
European Union	134.3	139.0
India	104.0	110.0
Russia	92.0	81.5
United States	44.9	45.2
Canada	33.8	37.0
Australia	39.0	29.0
Pakistan	26.4	26.8
Argentina	12.6	19.5
Turkey	17.3	19.0
Ukraine	20.9	16.5
United Kingdom	15.5	15.5
Iran	13.2	14.5
Kazakhstan	16.4	14.0
Brazil	10.4	10.0

Table 2: Wheat production in 2023-24 (USDA, 2024)

4.1.4. Peanut (groundnut) meal

Peanut meal, a by-product of peanut oil extraction (figure 2 shows production data), contains 45% protein and less than 10% fiber, making it a common choice in livestock and aquaculture feeds. It can replace costly ingredients like soy meal and fishmeal (FM) in fish diets, reducing feed expenses. It consists essential minerals like calcium, phosphorus, magnesium, sodium, sulfur, cobalt,

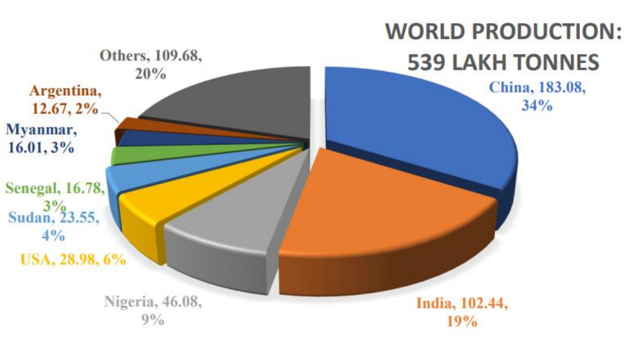

Figure 2: Groundnut production (indiastat.com, 2022)

copper, iodine, manganese, selenium, and zinc. These deficiencies impact livestock production, compromise animal health, and consequently, reduce farmers' income.

4.1.5. Molasses

Molasses is a significant byproduct derived from the processing of sugar beet (Beta vulgaris var. saccharifera), sugarcane (Saccharum L.), and citrus fruits, playing a crucial role in enhancing the economic value of these industries. It emerges as a residual substance following the primary crystallization process in sugar manufacturing. This viscous byproduct finds extensive use in various sectors including food processing, animal feed, and biofuel production. Its composition varies depending on factors such as the source of the plant material (sugarcane or sugar beet), plant maturity, and the methods used during processing. Derived primarily from sugar production, molasses is valued for its nutritional benefits, widespread availability, and cost-effectiveness, making it particularly popular in animal farming and aquaculture. Approximately 3.5 to 4.5 kg of molasses is produced for every 100 kg of processed plant material (n.d.). Although precise global production figures for molasses are challenging to ascertain, its production is closely linked with the scale of the global sugar industry, estimated at 1.83 billion tonnes in 2012 across 90 countries. This suggests significant global molasses production. Molasses offers several advantages including its iron content, use as a fertilizer, and its role as an energy source in fish feed and biofuel production. However, its high sugar content poses health risks for animals and contributes to greenhouse gas emissions. Therefore, a balanced approach considering both the benefits and drawbacks of molasses utilization is essential for its sustainable application. In aquaculture, molasses shows promise as a supplement, particularly in biofloc systems where its combination with cornstarch enhances shrimp growth and improves water quality. Its bioremediation properties further aid in reducing ammonia levels, thereby enhancing overall aquaculture productivity through improved water conditions. Table 4 presents the proximate composition of molasses.

Parameter	Content
Dry matter	53.1–82.5%
Protein	61.9 g/kg
Fat	4–20%
Ash	3–21.9%
Soluble sugar	237.5 g/kg
Total phenolic content	12.1 g/kg
Stachyose	34.2%
Saccharose	32%
Potassium	7.2%
Magnesium	0.7%
Phosphorus	0.58%
Chlorine	0.44%
Calcium	0.31%
Sodium	0.26%
Polyunsaturated fatty acids	61.2–63.4%
Linoleic acid	54–56%

Table 4: Proximate composition of molasses (Kassa et al., 2024)

4.1.6. Palm oil by-product: Palm oil mill effluent (POME)

Palm oil mill effluent (POME), a wastewater byproduct of oil palm processing, poses significant environmental challenges due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD). If left untreated, POME's nutrient-rich composition can lead to eutrophication, threatening aquatic ecosystems. However, research indicates the potential for repurposing POME in various beneficial applications. Studies suggest that POME can serve as an effective culture medium for microorganisms that are advantageous to aquaculture species, such as rotifers and fish larvae. Furthermore, POME has shown promise as a fertilizer for microalgae and a food source for chironomid larvae, highlighting its potential to contribute positively to aquaculture practices. Malaysia and Indonesia, producing 84% of the world's palm oil, are at the forefront of this industry, which is particularly significant in tropical countries like Malaysia, Thailand, and Indonesia. The palm oil industry consumes vast amounts of water and generates substantial quantities of wastewater, known as POME (Pauzi et al., 2023). Addressing the environmental impacts of POME through innovative reuse strategies could thus play a crucial role in sustainable aquaculture and environmental management in these regions.

4.1.7. Palm oil by-product: Palm kernel cake (PKC)

Palm kernel cake (PKC), a byproduct of palm oil extraction, is a viable protein and fat source for fish feed, effectively replacing traditional protein sources like soybean meal without compromising growth performance. Studies indicate that PKC inclusion levels up to 45% are safe for red tilapia, though higher levels may cause health issues like steatosis. For rohu, PKC levels up to 10% positively affect growth. However, excessive processing, such as fermentation to reduce fat content, can negatively impact fish growth. Thus, careful consideration of PKC inclusion levels and processing methods is essential for optimal fish health and growth.

4.1.9. Fruit and vegetable wastes

Fruit processing by-products, estimated at 100 million tonnes, pose significant management challenges but hold potential for aquaculture due to their bioactive compounds and enzymes. Incorporating 5% banana peel flour in fish feed benefits rohu (L. rohita) health. Orange peel enhances gilthead seabream (Sparus aurata) health when fed at 2.9 to 5.5 ppm of fish weight for 60 days. Chinese yam peel improves fish gut microbiota and counters pathogens like Vibrio and Pseudomonas. Pineapple waste, containing bromelain, boosts digestion and immunity in tilapia. Papaya waste, rich in papain, enhances growth performance and blood parameters in various fish species. Fruit and vegetable processing generates substantial waste: 1.81 million tonnes in India, 6.53 million tonnes in the Philippines, 32.0 million tonnes in China, and 15.0 million tonnes in the United States. Most of this waste is disposed of through composting or dumping, causing environmental pollution.

4.2. Fruit wastes as fish feed ingredients

Globally, 30-40% of apples are damaged and not marketed, while 20-40% are processed for juice extraction, leaving apple pomace, which can be used as livestock feed, containing 7.7% crude protein and 5.0% ether extract, providing 1.86 Mcal ME/kg DM and 1.06-1.12 Mcal NE/kg DM, with optimal feed conversion at 15% incorporation. Approximately 30-40% of banana production is rejected for quality but can be utilized as livestock feed, with banana peels constituting about 30% of fresh weight and banana leaves containing 10-17% CP. Citrus pulp, a residue from juicing 30% of citrus fruits, comprises 50-70% of the fruit's weight and can be fed to cattle or processed to avoid spoilage, containing 5-10% CP and 6.2% EE. Citrus molasses, a by-product of citrus juice extraction, contains 60-65% sugars and 4-5% CP, suitable for animal feed or silage. Grape by-products, including grape pomace and seeds, are rich in sugars and phenolics, with pomace containing 9-12% CP and 5-7% EE. Mango processing yields by-products such as mango kernel meal and peels, with the kernels containing tannins and other antinutritional factors. Pineapple processing results in skins, crowns, and pomace, with pineapple bran being fed fresh, dried, or ensiled, though it requires supplementation with protein and minerals due to deficiencies.

4.3. Vegetable wastes as fish feed ingredients

Baby corn, widely consumed in Asian cuisine, Thailand as the leading producer and exporter, with India emerging due to its low production costs and surging cultivation to meet demand, yielding 7.5–8.7 tonnes/ha. With only 15% of baby corn being the edible cob and the rest 85% being husk, it poses environmental concerns, though by-products like tassels and green plant material can provide additional income if effectively managed. Carrot by-products, including cull or surplus carrots, tops, and pomace, can be used as livestock feed either fresh, ensiled, or dehydrated. Fresh carrots are rich in vitamin C (300–700 mg/kg DM), carotene, and contain 10% CP and 1.4% EE. Pea by-products, including pea vine, straw, and pods, offer nutritious feed options with high palatability and digestibility, containing significant amounts of CP and EE, making them suitable for ruminants. Surplus and cull potatoes, while unpalatable raw and potentially harmful due to solanine in sprouts, can be fed to livestock if boiled or steamed, containing 65–75% starch and 9.5% CP. Tomato waste, including culled tomatoes and pomace, provides valuable feed components with tomato pomace being rich in residual nutrients and posing no risk from tomatine once ripened.

4.4. Leaf meal as fish feed ingredients

Plant-based protein sources in fish feed support sustainable aquaculture, leveraging their availability and cost-effectiveness. Various plant leaves like turi, drumstick, ipil-ipil, and others such as alfalfa, mulberry, and sweet potato are utilized in aquafeed. These ingredients are used fresh, dried, powdered, cooked, fermented, or as concentrates. They serve in direct feeding of herbivorous fish and as supplements or partial substitutes for fishmeal in aquafeed formulations, enhancing feed efficiency and sustainability in the aquaculture sector.

Leaf meals for fish feed originate from both terrestrial and aquatic plants (Bardach et al., 1972), categorized into:

1. Leaf meals from terrestrial plants
2. Leaf meals from aquatic plants

4.4.1. Leaf meals derived from terrestrial plants

Plant-based ingredients in aquafeed pose challenges due to antinutritional factors (ANFs). Techniques like heat treatments, soaking, fermentation, and enzyme supplementation help reduce ANFs in leaf meals. These ingredients also contain non-starch polysaccharides (NSPs), which fish struggle to digest due to enzyme deficiencies. Exogenous carbohydrases effectively lower NSP levels in plant-based feeds, enhancing digestion, growth, and nutrient utilization in fish.

4.4.1.1. Hygrophila spinosa leaf meal

Hygrophila spinosa (*Hygrophila auriculata*), a marsh-loving herbaceous plant in the Acanthaceae family, is native to tropical Asia and Africa, including India, Sri Lanka, Myanmar, China, Malaysia, Burma, Bangladesh, Nepal, and Pakistan. Known as gokulakanta, its leaf meal (HSLM) boasts 20.6% crude protein, 36.4% nitrogen-free extract, and significant mineral (18.3%) and crude fiber (21.3%) content. Despite containing anti-nutritional factors like phytosterols, tannins, flavonoids, terpenoids, and NSP, which limit its use in monogastric animal diets, especially fish, substituting H. spinosa leaf meal for deoiled rice bran (DORB) in fish feed remains unexplored. Supplementing the leaf meal with exogenous carbohydrases may alleviate the NSP's adverse effects.

4.4.1.2. Sweet potato leaf meal

Treatment	Particulars	Raw(0 hr)	Soaked(24 hr)	Fermented(120 hr)
Proximate composition	CP (%)	$23.9^b \pm 0.1$	$23.4^b \pm 0.42$	$30.3^a \pm 0.48$
	CL (%)	5.55 ± 0.68	5.14 ± 0.52	7.05 ± 0.41
	CF (%)	8.30 ± 0.19	8.10 ± 0.19	6.01 ± 0.75
	ASH (%)	10.17 ± 1.01	9.60 ± 1.45	10.21 ± 1.45
	NFE (%)	52.04 ± 0.96	53.86 ± 1.35	46.36 ± 2.10
Antinutritional factor	Tannin (mg/100 g)	$23.03^a \pm 0.94$	$4.84^c \pm 0.56$	$8.53^b \pm 0.14$
	Try (TIA mg/g	3.20 ± 0.46	2.50 ± 0.22	2.90 ± 0.16
	Phy (mg/100 g)	$15.02^a \pm 0.68$	$11.06^b \pm 0.05$	$9.38^c \pm 0.10$
	Oxalate (%)	$1.36^a \pm 0.01$	$0.29^c \pm 0.007$	$0.94^b \pm 0.01$
	Alkaloid (mg/g)	$1.36^a \pm 0.14$	$1.00^b \pm 0.17$	$0.73^b \pm 0.06$
	HCN (mg/100 g)	$38.8^a \pm 1.24$	$23.0^b \pm 1.90$	$23.04^b \pm 1.9$

Note. CF, crude fibre; CLcrude lipid; CP, crude Protein; HCN, hydrogen cyanide; NFE, nitrogen-free extract; Phy, phytate; Try, trypsin inhibitor.
Data represent mean ± S.E. Values in the same column with different superscripts are significantly different ($p < 0.05$), $n = 3$.

Table 5: Proximate composition&ANF of sweet potato leaf meal (Meshram et al., 2018)

Ipomoea batatas leaf is a valuable protein and energy source in fish diets. Sweet potato leaf meal (SPLM), with its high protein content (23%-33%) and favorable amino acid profile, benefits animal diets. However, antinutritional factors (ANFs) like phytate, trypsin inhibitor, alkaloid, oxalate, tannin, and cyanide limit its use by reducing digestibility and growth. Methods like soaking and fermentation help mitigate ANFs, with fermentation notably enhancing nutrient digestibility and availability. Recent studies show the effectiveness of fermented ingredients in poultry, piglet, and fish diets. Methods like moist heat, water soaking, and fermentation neutralize antinutritional factors (ANFs) in leaf meals. Fermentation with microorganisms innovatively reduces ANFs and digests crude fiber in sweet potato leaf meal (SPLM). Fermented leaf meal is used in chicken, piglet, and fish feeds. Solid-state fermentation (SSF) detoxifies and enhances the nutritional value of agro-industrial by-products, reducing crude fiber. This study aims to find the optimal level of DORB substitution with fermented SPLM without affecting growth, survival, and feed efficiency in L. rohita fingerlings.

4.4.1.3. Sesbania aculeata leaf meal

Sesbania aculeata (dhaincha), found in tropical Asia and Africa, has leaf meal containing 22%-30% crude protein. However, its seeds have antinutritional factors (ANFs) like saponin, tannins, and trypsin inhibitors, which must be reduced or removed for feed use. Economical processing techniques are essential. Solid state fermentation (SSF) has been effective in incorporating plant ingredients into diets for chickens, pigs, and fish. SSF detoxifies tannin and phytate in seeds and improves nutritional value by reducing ANFs and enhancing nutrient availability.

4.4.1.4. Berseem (Trifolium alexandrinum) leaf meal

Berseem (Trifolium alexandrinum) leaf meal is a promising fish feed due to its high crude protein content. Domesticated in Egypt, it is now widely grown in West and South Asia, with India leading in cultivation. Berseem is nutrient-rich, containing 15-25% protein, 11-19% minerals, and carotene, with 70% dry matter digestibility in ruminants. Its leaves have 23.36% crude protein, 3.16% ether extract, 17.04% crude fiber, and 19.19% total ash. Traditionally used in cattle, pig, and poultry diets, recent studies show that 5-10% berseem in Tilapia diets improves growth and protein efficiency ratio (PER).

4.4.1.5. Mucuna Leaf Meal (Mucuna bracteata)

Mucuna bracteata, a leguminous plant, plays a vital role in agroecosystems where rubber trees, oil palm, citrus, and coconut thrive. It acts as a nitrogen-regulating cover crop, protecting soil from weeds, enhancing fertility through nitrogen fixation, and preventing erosion. Originally from Northeast India, it's integrated into Hevea rubber plantations in India and oil palm estates in Malaysia. Thriving in warm, humid climates with temperatures of 20-35°C and consistent rainfall, M. bracteata grows rapidly, up to 10-15 cm per day, similar to rubber and palm oil plants. Beyond agronomic benefits, it improves soil health and water retention, supporting sustainable agriculture in plantation ecosystems.

Mucuna Leaf Meal (Mucuna bracteata) is a nutrient-rich feed ingredient with 28.12% crude protein, 2.63% crude lipid, 17.69% crude fiber, 44.79% nitrogen-free extract, and 18.76% ash. It provides a gross energy of 370.61 kcal/100g. Despite its nutritional benefits, it contains antinutritional factors such as tannins (21.16 mg/100g), alkaloids (2.40 mg/100g), phytic acid (13.76 mg/100g) and saponin (13.37 mg/100g).

4.4.1.6. Crassocephalum crepidioides leaf meal

Crassocephalum crepidioides, known as Terapaibee in Manipuri, is a nutritious plant found abundantly in India's North-East region, particularly Manipur. This perennial herb belongs to the Asteraceae family and is highly adaptable to harsh environments while displaying strong disease resistance. Fireweed, as it is commonly called, is a wild and underutilized vegetable that offers a rich source of micronutrients and natural antioxidants. It boasts a high protein content of 27% with all essential amino acids and contains minerals such as sodium, potassium, phosphorus, magnesium, calcium, iron, manganese, and copper.

4.4.1.7. Black gram (Vigna mungo) leaf meal

The Fabaceae family includes various gram varieties, with black gram (Vigna mungo), commonly known as urad dal, being particularly significant in tropical and sub-tropical agriculture. This crop thrives in challenging climates and enhances soil fertility. Black gram foliage is rich in crude protein, approximately 15.5%. Despite its nutritional value, the presence of anti-nutritional factors like phytic acid, saponin, and polyphenols limits its direct use in aquafeed. This study seeks to determine the optimal inclusion level of black gram leaf meal (BGLM) as a replacement for deoiled rice bran (DORB) in the diet of *Labeo rohita* fingerlings.

4.4.1.8. Pigeon pea (*Cajanus cajan*) leafmeal

Pigeon pea (Cajanus cajan L.) is a cost-effective protein and energy source in many third-world countries, including India. Widely cultivated, it plays a significant role in global production. Consumed mainly as dhal, pigeon pea is nutrient-dense but contains antinutritional factors that can be mitigated through different processing methods. This study investigates the use of pigeon pea leaves as a substitute for DORB. The proximate contains 4.80% moisture content, with the remaining 95.20% as dry matter. Within the dry matter, crude protein levels are 19.97%, ether extract (fat) is 3.23%, crude fiber comprises 16.55%, and ash makes up 7.65%. Non-fibrous carbohydrates (NFE) constitute the majority of dry matter at 52.61%. These findings offer a detailed overview of the nutritional composition of the sample.

4.4.1.9. Green pea (*Pisum sativum*) leaf meal

Green pea, Pisum sativum leaf meal (PSLM), contains approximately 220–260 g crude protein/kg, 20–30 g crude lipid/kg, and 50–110 g crude fiber/kg. While PSLM has been used successfully in livestock feed, its use in fish diets is less studied. Research suggests PSLM can be included at 200 g/kg in aquafeed for Chanos chanos. However, ANFs such as trypsin inhibitors, phytic acid, lectin, tannins, saponin, cyanogens, and anti-vitamins can hinder fish growth and reduce feed efficiency. Efforts to improve P. sativum leaf meal utilization in Labeo rohita diets, including mitigating ANFs like NSPs, are limited. This study aims to optimize P. sativum leaf meal as a DORB alternative in L. rohita fingerling diets using exogenous cellulase and xylanase mixtures.

4.4.1.10. Hygrophila spinosa leaf meal

Hygrophila spinosa, a forage plant from the Acanthaceae family, grows in marshy areas in tropical Asia and Africa. Known as gokulakanta, it is found in India, Sri Lanka, Myanmar, China, Malaysia, Burma, Bangladesh, Nepal, and Pakistan. Hygrophila spinosa leaf meal (HSLM) contains 20.6% crude protein, 36.4% nitrogen-free extract, 18.3% minerals, and 21.3% crude fiber. It also has anti-nutritional factors like phytosterols, tannins, flavonoids, terpenoids, and NSP, which limit its use in monogastric animal feed, including fish. Its use as an alternative to DORB in fish diets is unexplored. Adding exogenous carbohydrases can reduce NSP's negative effects. Exogenous NSP-degrading enzymes like cellulase and xylanase disrupt plant cell walls, reducing NSP size, enhancing digestion, and improving nutrient utilization and growth in fish.

4.4.1.11. Turi leaf meal

Sesbania grandiflora, or turi, is a robust tropical shrub grown extensively in Africa and Asia. It thrives in varied conditions including waterlogged and saline soils, making it valuable for fodder, green manure, and aquafeed due to its high protein and low fiber content. Though rich in essential fatty acids, turi lacks essential amino acids except for leucine, tryptophan, and histidine. Its crude protein content (22-30%) surpasses that of conventional legumes like chickpea, mungbean, and cowpea. Studies confirm that adding turi leaf meal to diets promotes growth in fish species such as Labeo rohita and Orechromis niloticus.

4.4.1.12. Moringa leaf meal

Fresh Moringa oleifera leaves, known as 'drumstick', are highly nutritious supplements for plant-eating fish like tilapia, barbs, and fancy carps. They are rich in protein, lipids, vitamins, and minerals, making them valuable in aquaculture feeds. Processing methods such as drying, soaking, and grinding help reduce anti-nutritional factors, which are generally low in Moringa leaves except for saponins and phenols. Moringa leaves provide essential amino acids like methionine, cystine, and tryptophan, promoting fish growth and health. Studies show that Moringa leaves can partially replace conventional diets without compromising growth performance in species like Nile tilapia, common carp, and African catfish. However, caution is advised with high inclusion levels of Moringa leaf meals due to potential costliness and the risk of compromised growth and health.

4.4.1.13. Subabul leaf meal

Leucaena leucocephala, or ipil-ipil, is a fast-growing tropical leguminous plant used widely in animal feed. Its leaves contain over 20% crude protein (dry matter basis), and the seeds are rich in α-carotene with a favorable amino acid profile. However, the toxic non-protein amino acid mimosine can retard animal growth, limiting its use. Despite this, studies show improved growth in tilapia with leucaena leaf meal in their diet and benefits for small fish species like mollies, topminnows (Poecilia spp.), and Macrobrachium rosenbergii. In Oreochromis niloticus fingerlings, leucaena leaf meal at 33% to 100% inclusion enhanced growth under cage farming conditions, though levels above 25% negatively impacted growth. Additionally, leucaena seeds are known to enhance growth and survival in Clarias gariepinus.

4.4.1.14. Alfalfa leaf meal

Alfalfa (Medicago sativa) is widely valued in cattle forage due to its high protein content, balanced amino acid profile, and richness in vitamins and carotenoids. Both fresh and dried alfalfa leaves have demonstrated positive outcomes when included in tilapia diets. Research indicates that including alfalfa leaf protein up to 35% in tilapia diets supports growth and survival. In common carp and mrigal, inclusion of alfalfa leaf meals up to 40% and 30%, respectively, has enhanced growth, survival rates, and increased protein and lipid content. Dehydrated alfalfa leaves have also been successfully utilized in tilapia diets. However, to optimize growth performance, researchers advise careful inclusion levels.

4.4.1.15. Mulberry leaf meal

Morus alba, or mulberry leaves, grown for silkworms, are used in fish feed due to their high protein and mineral content. Despite deficiencies in essential amino acids and the presence of anti-nutritional factors, fermentation can reduce these issues cost-effectively. Mulberry leaf meal has proven to be a promising protein source for Labeo bata. For Nile tilapia, a diet with 60% mulberry leaf meal and 40% rice bran enhances growth and feed conversion ratio. In stinging catfish, mulberry leaf meal improves growth, survival, fish acceptance, and immunity.

4.4.1.16. Sweet potato leaves

Sweet potato (Ipomoea batatas) is a globally cultivated key food crop, especially in tropical regions. Its protein- and fiber-rich leaves serve as cattle forage, containing 26.5-32.5% crude protein and significant amino acids, minerals, and vitamins. Sweet potato leaves, harvested year-round, are cost-effective for fish feed. Processing methods like drying, boiling, steaming, and grinding improve palatability and remove anti-nutritional factors. Research on Tilapia zilli shows that up to 15% sweet potato leaf meal in diets maintains growth and feed efficiency.

4.4.1.17. Cassava leaf meal

Cassava leaf, known for its palatability and high protein content, contains approximately 16.7% to 39.9% crude protein and minerals. Methods such as soaking and sun-drying are used to reduce anti-nutritional factors like cyanogenic glycosides and tannins. Research indicates that cassava leaf meal can be included in Nile tilapia diets without compromising growth and survival. It has also been shown to replace up to 20% of African catfish diets without negatively affecting growth and nutrient utilization.

4.4.2. Leaf meals derived from aquatic plants

Aquatic macrophytes serve multiple purposes, including food, medicine, and livestock fodder. Their nutrient-rich leaves are cost-effective, readily available, and nutritionally dense. Used as fish feed, aquatic weeds benefit herbivorous and omnivorous fish like grass carp, common carp, tilapia, and amur carp, reducing feed costs and promoting eco-friendly practices. Aquatic plants also offer alternative protein sources, successfully replacing fish meal in some studies.

4.4.2.1. *Houttuynia cordata* leaf extract and meal

Houttuynia cordata, from the Saururaceae family, is native to Southeast Asia. This herb is used traditionally for its antiviral, antibacterial, immunostimulant, diuretic, anti-cancer, and anti-inflammatory properties. Studies show that dietary H. cordata enhances lysozyme, phagocytic, and superoxide dismutase activities, while reducing ROS in cobia (Rachycentron canadum). Its immunological benefits are attributed to bioactive compounds such as flavones (e.g., quercitrin, kaempferol), essential oils (e.g., houttuynin, limonene), and alkaloids (e.g., Aristolactam A, splendidine). Additionally, 346 volatile components have been identified in H. cordata. Despite the known immunostimulatory properties of various plant extracts, their molecular mechanisms in animal immunity, including fish, remain largely unexplored.

4.4.2.2. Azolla

Azolla, or mosquito plant, is a fast-growing aquatic fern known for its nitrogen-fixing ability and nutrient-rich composition, including essential amino acids, vitamins (A, B12, Beta-Carotene), and minerals crucial for growth. Its protein content ranges from 19% to 31% with low lignin, making it valuable in animal feed, especially in aquaculture. While direct feeding of fresh or dried Azolla to fish can sometimes hinder growth, using Azolla as a protein source in fish diets has proven beneficial, enhancing feed efficiency and growth in species like tilapia, Rohu, and common carp. Recommended inclusion levels vary, with suggestions from replacing up to 25% of fishmeal in tilapia diets to higher percentages in other fish species' diets. Overall, Azolla offers a sustainable and economical option for aquafeed, supporting environmentally friendly aquaculture practices.

4.4.2.3. Water hyacinth

Water hyacinth (Eichhornia crassipes), native to the Amazon basin, is a rapidly growing, invasive aquatic plant. It contains 38% protein and 17-26% minerals, making it useful as animal feed. For fish, composted or fermented water hyacinth is recommended. Fermented hyacinth can make up 25% of Nile tilapia diets, improving palatability and utilization, while composted hyacinth can replace 75% of fishmeal. Up to 18.9% dried hyacinth is suitable for Matrincha fish, but higher levels are not advised due to tannin content.

4.4.2.4. Duckweeds

Duckweeds, floating aquatic plants in tropical and subtropical freshwater, contain 15-43% protein, 5-30% fiber, and 5% lipids, making them suitable for animal feed. Fresh duckweed is fed to fish like common carp, Thai sharputi, raj puthi, silver carp, mrigal, and tilapia. It can supplement up to 15% of common carp diets to reduce costs. Dried duckweed suits Labeo rohita fingerlings, while fermented lemna leaf meal can replace up to 20% of commercial fish meal for common carp. Solar-dried duckweed can replace up to 30% of fish meal for Nile tilapia, and 10% enhances growth and survival of mango tilapia fingerlings.

4.5. Non edible seeds

4.5.1. Jatropha kernel meal

Jatropha curcas kernel meal, a biodiesel byproduct, shows promise as a sustainable fish feed ingredient due to its high protein content. However, raw Jatropha contains antinutritional factors like phorbol esters, necessitating detoxification before use in feed (Nutritional evaluation of Jatropha protein concentrate and fermented Jatropha protein concentrate in Labeo rohita fingerlings, n.d.). The proximate composition of Jatropha kernel meal varies but typically includes crude protein levels similar to fish meal (around 60%) (Substitution of fish meal by Jatropha curcas kernel meal: effects on growth performance and body composition of white leg shrimp (Litopenaeus vannamei), 2011). Studies indicate that detoxified Jatropha kernel meal can partially replace fish meal in diets for fish species like Labeo rohita and Cyprinus carpio without compromising growth (Substitution of fish meal by Jatropha curcas kernel meal: effects on growth performance and body composition of white leg shrimp (Litopenaeus vannamei), 2011). Further research is necessary to optimize detoxification methods and determine appropriate inclusion levels for various fish species

4.5.1. Cotton seed

Cottonseed ranks as the third most significant plant-based protein source by weight, following soybean and rapeseed, and is utilized globally due to its relatively lower cost compared to animal proteins. The National Cottonseed Production Association (NCPA) indicates that approximately 450 kilograms of cottonseed meal can be derived from one ton of crushed cottonseed. There are two primary methods for extracting cottonseed meal: mechanical extraction and solvent extraction. Regardless of the method used, the resulting meal must contain a minimum of 36 percent crude protein.

Proximate Composition of Cottonseed meal Solvent Extracted	
International Feed Number 5-01-619	
Typical Dry Matter	92%
Crude Protein	41.7%
Crude Fat	1.8%
Crude Fat	11.3%
Ash	6.4%
Amino Acid Composition	
Arginine	3.97%
Histidine	0.83%
Isoleucine	1.15%
Leucine	1.8%
lysine	1.8%
Methionine	0.5%
Cystine	0.45%
Phenyl alinine	2.1%
Tyrosine	0.8%
Threonine	1.02%
Tryptophan	0.42%
Valine	1.68%
Mineral Composition	
Calcium (%)	0.17
Phosphorus (%)	1.17
Potassium (%)	1.39
Chlorine(%)	0.04
Magnesium (%)	0.41
Sodium (%)	0.04
Sulphur (%)	0.30
Copper (mg/kg)	19.00
Iron (mg/kg)	208.0
Magnesium (mg/kg)	21.0
Selenium (mg/kg)	0.06
Zinc (mg/kg)	61
Vitamin Composition	
Biotin	0.97mg/kg
Choline	2764 mg/kg
Folacin	1.4 mg/kg
Niacin	41 mg/kg
Pantothenic acid	13.7 mg/kg
Pyridoxine	7 mg/kg
Riboflavin	3.3 mg/kg
Thiamine	6.6 mg/kg
Vitamin B12	-
Vitamin E	16 mg/kg
Vitamin K	-

The proximate composition of cottonseed meal is detailed in Table 6. Given its high protein content and cost-effectiveness compared to other legumes and fishmeal, cottonseed meal (CSM) holds substantial potential for inclusion in high-protein aquaculture feeds. Nutritionally, cottonseed meal is rich in protein and highly palatable to fish. The inclusion rate of CSM in animal feeds is influenced by factors such as the species of animal, developmental stage, dietary protein requirements, available lysine, and the presence of anti-nutritional factors. The acceptable levels of CSM in fish feeds vary widely among species, including tilapia (Sarotherodon mossambicus), Nile tilapia (Oreochromis niloticus), channel catfish (Ictalurus punctatus), Chinook salmon

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Proximate composition	SFSM
Dry matter (%)	96.24
Protein (%)	19.42
Lipid (%)	8.76
Fibre (%)	-
Ash (%)	2.82
NFE (%)	62.68
EAA (g 100g^{-1} DM)	
Arginine	1.68
Histidine	0.81
Isoleucine	0.51
Leucine	1.21
Lysine	0.81
Methionine	0.13
Phenylalanine	0.93
Threonine	0.64
Valine	0.94

(Oncorhynchus tshawytscha), coho salmon (Oncorhynchus kisutch), rainbow trout (Oncorhynchus mykiss), and rohu (Labeo rohita).

4.5.2. Sunflower Seed Meals (SFSM)

Increased sunflower seed production for oil can yield more meal, potentially replacing costly soybean meal. Sunflower seeds grow worldwide due to their adaptability to various climates and soils. Sunflower seed meal (SFSM), a by-product of oil extraction for human consumption, varies in quality due to processing methods and seed types. Efficient methods can produce SFSM with 42-46% protein and 8-14% cellulose. De-hulling and oil extraction processes influence protein content (29-45%) inversely related to fiber (14-32%)(Table7).

Table 7: Proximate Composition of Sunflower Seed Meals (SFSM)

4.5.3. Neem seed

Azadirachta indica Juss, a member of the Meliaceae family, is an evergreen tree commonly known as neem, originating in India. A. indica is endemic to the Indian subcontinent and has been extensively introduced throughout tropical and subtropical regions. It is a multipurpose tree well known for its commercial and medicinal uses. Its durable wood and various parts like flowers, fruits, seeds, oil, cake, leaves, bark, and gum are utilized for their medicinal properties. A fully grown tree produces 30-100 kg of fruits depending on rainfall, and about 60% of neem fruits go uncollected. Neem seed yields approximately 20% oil and 80% seed cake. Neem oil is highly valued for its medicinal properties, with seed kernels containing 40-48.9% oil, providing substantial commercial value. The extraction of neem oil for biodiesel production results in a significant quantity of neem seed cake (NSC) rich in true protein. NSC from whole seeds contains 12-20% protein, while decorticated seed cake can contain 34-41% protein, along with a balanced amino acid profile containing essential amino acids.

4.5.4. Canola (Rapeseed)

	Rapeseed seeds	Rapeseed cake	Rapeseed meal	Rapeseed concentrate	Rapeseed isolate
Nutrient composition (% DM)					
Dry matter	87.3–94.9	92.6–94.9	71.0–99.2	91.5–96.4	94.6–97.8
Crude protein	17.5–24.6	29.4–38.4	30.0–60.6	48.3–80.6	81.2–98.7
Crude fat	39.8–51.0	10.3–20.3	0.1–17.6	0.3–9.5	0.9–4.1
Crude Fibre	5.8–15.7	8.3–19.7	1.4–36.1	0.5–4.9	<0.01–2.1
NfE	-	25.2–30.5	15.9–54.3	11.6–42.5	<0.01
Ash	3.7–5.0	5.5–6.7	3.8–15.1	5.9–20.8	0.4–6.8
Total phosphorus	0.6–0.8	0.2–1.1	1.1–2.4	0.9–3.6	-
Amino acid composition (% protein)					
Arginine	5.6–7.3	5.6	3.1–8.4	3.4–7.6	6.7–7.7
Histidine	2.6–3.5	2.5	1.6–5.4	2.0–3.9	2.4–2.9
Isoleucine	3.9–5.6	3.6	2.6–4.5	3.4–4.4	3.7–5.0
Leucine	6.3–9.5	6.4	3.8–9.2	6.7–8.0	6.4–8.9
Lysine	5.8–6.8	5.0	3.4–6.9	4.8–7.9	3.3–5.4
Methionine	1.8–2.1	1.9	0.6–2.4	1.9–2.1	1.7–2.0
Cysteine	2.3–2.8	2.2	1.2–2.9	4.2–5.2	1.2–4.1
Phenylalanine	3.6–5.8	3.6	3.3–5.2	3.6–4.5	4.2–5.3
Threonine	4.2–6.6	4.2	2.1–5.8	4.2–4.4	3.2–4.3
Tryptophan	1.3	1.4	0.6–1.6	1.4	1.5–1.6
Valine	4.9–7.0	4.7	2.3–6.1	3.7–5.4	4.5–5.9
Anti-nutritive factor content					
Glucosinolates (μmol/g)	5.88–31.21	-	<0.1–129.2	<0.1–3.4	<0.1–2.0
Phytic acid/phytate (%)	2.0–4.0	-	0.1–6.1	<0.1–2.3	0.2–2.3
Tannins (%)	0.0–14.4	1.3–2.0	0.1–4.3	<0.01–<0.1	-
Sinapine (%) (+ sinapic acid [%])	-	-	<0.01–2.6	0.02	0.1

Table 8: Proximate composition of Canola (Rapeseed)

Canola meal is regarded as a valuable source of vegetable protein for the livestock industry. The primary nutrient components of canola meal include protein, carbohydrates, crude fiber, and ash, along with residual oil that remains after the oil extraction process (see Table 8). Although canola meal is widely used as a protein source in animal diets, it has a lower protein content (36%) and available energy (2,000 kcal/kg AME) but a higher fiber content (12%) compared to soybean meal, which contains 7% fibre.

4.5.5. Safflower oil seed meal

Safflower, an adaptable oleaginous plant from the Asteraceae family, is grown in nearly 60 countries worldwide, including Kazakhstan, India, Mexico, and the United States. Safflower oilseed meal contains moderate levels of protein (19.42%), crude lipid (8.76%), ash (2.82%), and nitrogen-free extract (62.68%). The seed composition typically includes 33-60% coat and 40-67% food stores, with oil content ranging from 15% to 45% depending on variety and environment. Used in animal feeds and fish diets, safflower meal can constitute up to 20% of protein sources in feeds for rainbow trout and shrimp. Beyond agriculture, safflower finds applications in pharmaceuticals, infant formulas, cosmetics, and biodiesel production. Known for its taproot, safflower efficiently utilizes nitrogen leachates, making it environmentally friendly by reducing groundwater contamination.

4.5.6. Hemp Seed

Hemp (Cannabis sativa L.) is an herbaceous annual from the family Cannabinaceae, originating in Eurasia. Cultivated for over six millennia, it serves various purposes: food, fibre, and medicine. Classified as marijuana or hemp based on THC content (0.2% in Europe, 0.3% in North America), hempseed contains 26–37.5% lipids, 25% crude protein, and 28% fibre; hempseed cake, 11% lipids, 33% crude protein, and 43% fibre. Traditionally used in bird feed, whole hempseeds are costly, but improved practices may make them economical for livestock feed. Hempseed cake, with 10% oil, serves as nutritious livestock feed. Residual material from oil extraction is used as fertilizer or compressed into seedcake. Hempseed is also used as fishing bait in France, Scotland, and North America. Table 9 shows proximate Composition of hemp deed

Composition (%)	Crude oil	Crude protein	Crude fibre	Ash
Whole seed[a]	35.5	24.8	27.6	5.6
Seed meal	11.1	33.5	42.6	7.2
Whole seed[b]	30.4	24.0	32.1	4.8
Seed meal	10.2	40.7	30.5	6.7

Table 9: Proximate composition of Hemp seed

4.5.7. Rubber Seed Cake

Rubber seed, a key biomass component of Hevea brasiliensis, originates from South America and has been cultivated in Southeast Asia since 1876. Comprising 65% kernel and 35% shell, rubber seed yields range from 150-250 kg per hectare, influenced by soil nutrients, ecosystem, materials used, and crop density. Primarily grown for latex, which is globally used in industrial and domestic products.

Moisture content: Rubber seed moisture ranges from 1.5% to 2.8%, impacting shelf life and microbial susceptibility.

Total ash (TA): Typically, below 2.5%, with most between 3-5%, crucial for diet compounding.

Crude fat: Recent studies report 45.50%, impacting digestibility and palatability.

4.6. Micro and macroalgae

Algae, both microscopic (microalgae) and macroscopic (macroalgae), are a valuable alternative feed ingredient in aquaculture. Adaptable to diverse environments, algae are rich in protein, carbohydrates, lipids, and beneficial biomolecules like astaxanthin, lutein, and omega-3 fatty acids, promoting fish health. Algae-derived omega-3s are preferred over fish oil due to their purity, lack of off-flavors, low cholesterol, and cost-effectiveness. Red seaweeds hold promise due to their high protein content. Table 10 shows the species used as feed ingredient

Species	Protein	Carbohydrates	Lipids
Anabaena cylindrica	43-56	25-30	4-7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	3
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirogyra sp.	6-20	33-64	11-21
Arthrospira maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechococcus sp.	63	15	11

Table 10: General composition of different algae (% of dry matter)
Source: Maisashvili *et al.* (2015)

.4.7. Single cell proteins

Single-cell protein, derived from microorganisms like yeast, fungi, algae, and bacteria, is a promising alternative protein source for fish feed. SCP production offers rapid growth, minimal land use, and weather resilience. Yeasts, especially Saccharomyces cerevisiae, Candida utilis, and Kluyveromyces marxianus, are prized for their amino acid profiles and high protein content (40-50%), enhancing fish growth, antioxidant defenses, and digestive enzyme activity. SCP production from low-value lignocellulosic biomass and waste streams offers a sustainable method for generating protein-rich fish feed.

5. Animal-based by-products

5.1. Blood meal

Blood meal, a nutrient-rich byproduct from animal processing, contains roughly 90% protein, 3% lipids, and 4% ash. While deficient in isoleucine, it offers a good profile of other essential amino acids. Studies show blood meal can replace up to 50% of fishmeal in Nile tilapia diets without adverse effects. However, research across various fish and shrimp species suggests that while blood meal can be a viable fishmeal substitute, its efficacy varies by species, with lower inclusion levels generally yielding better results.

5.2. Poultry by-product meal (PPM)

Poultry by-product meal (PPM), derived from poultry slaughterhouses and processing plants, includes poultry offal meal (POM) and feather meal used in animal and fish feed. Studies explore PPM's potential to replace fishmeal (FM) in aquaculture diets for various species. It has been found that PPM can substitute up to 40% of FM without compromising European seabass fry growth, but adverse effects were observed on juvenile barramundi. Combining insect meal with PPM shows promise in gilthead seabream, while total PPM replacement in freshwater fish feeds remains less feasible. Fermented PPM and enzymatically treated feather meal enhance feed formulations for species like Nile tilapia, tiger shrimp, silver pompano, largemouth bass, and rainbow trout.

5.3. Fish processing wastes

Fish processing generates significant waste, including parts unsuitable for direct consumption. Some of this waste is converted into fishmeal (FM) for pig, poultry, and aquaculture feeds, but not all can be utilized this way. Additionally, fish processing produces valuable by-products like skin, bones, and shellfish remnants, which are rich in nutrients such as gelatin, collagen, antioxidants from bones, and methionine and lysine from shellfish waste. Despite these benefits, FM remains the preferred fish processing waste for aquaculture and other feeds. Further research is needed to explore the potential of other fish processing wastes for aquafeed in the future.

5.4. Chicken manure

Chicken manure is widely used in aquaculture to boost microalgae and zooplankton growth, and as feed for fish. It enhances fish growth in Nile tilapia ponds and Tilapia rendalli farming. Fermented chicken manure appears safer than non-fermented types, yet concerns about heavy metals and coliform bacteria remain. Disinfection is crucial for safe aquaculture products.

5.5. Pig manure

Swine by-products, including fresh pig manure, enhance fish growth and water quality in aquaculture. Fresh pig manure is recommended for carp farming in Northern Italy due to improved survival rates and production with commercial pellets. It enriches phytoplankton and zooplankton in ponds and can replace up to 15% of fishmeal in Nile tilapia feed, benefiting aquaculture species directly when applied to fish ponds.

5.6. Insect meals

Insects, making up 70–75% of Earth's animal species, recycle materials in ecosystems. They convert feed efficiently, breed on bio-waste, and emit fewer greenhouse gases than livestock. Commercializing insect protein is still new. *Hermetia illucens* (Black Soldier Fly), mealworms, and maggots can replace traditional fish meal in aquaculture, offering essential amino acids. Insects are rich in protein and lipids but pose a challenge with chitin content. Supplementing with omega-3 from fish offal can address their lower fatty acids. Studies show whole insects effectively feed various fish species. More research is needed on digestibility, toxins, and optimal insect diets. Insects could replace fish meal and soy-based diets in farming due to their nutritional and eco-friendly benefits.

6. Advantages of incorporating sustainable waste in aquafeed

Using sustainable agricultural waste in aquafeed benefits both the aquaculture industry and fish health. By-products like blood meal and poultry waste make production more economical and eco-friendlier, improving waste management and reducing resource use. This method supports fish health by providing essential nutrients and boosting immune systems, thus reducing disease susceptibility. Agricultural waste-derived probiotics enhance fish immune responses, leading to healthier stocks. As the industry aims to produce 109 million tonnes of aquatic protein by 2030, these practices are crucial for sustainable growth and fish welfare.

7. Challenges of using agricultural waste in aquafeed and aquaculture practices

Using plant-based agricultural waste in aquafeed is challenging due to anti-nutritional factors (ANFs) and high cellulose content, which reduce nutritional quality. Methods like soaking, boiling, fermentation, and genetic manipulation mitigate ANFs without compromising nutritional value. Fermentation improves nutrient bioavailability, but its full potential in aquaculture is not fully understood. Low levels of ANFs can benefit animal health, but pesticide residues and synthetic antibiotics in waste pose environmental and fish safety risks. More research is needed to evaluate these risks in aquaculture settings.

8. Conclusion and recommendations

In conclusion, agricultural wastes offer nutritional benefits and cost reductions for aquaculture but require proper management to avoid environmental and health risks. Transforming these by-products into valuable resources is crucial for community benefit, sustainability, and affordable aquaculture. Future studies must address risk assessments and economic evaluations to integrate agricultural waste effectively into aquaculture.

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Chapter: 2

Feeds and Feeding strategies towards a Sustainable Shrimp Crop

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1. Introduction

Nutrition is the science of identifying, quantifying and supplying an animal the essential nutrient substances required to perform its normal physiological functions – including maintaining a highly effective natural immune system, growth and reproduction (Doe, 2022). Feed is the form of ingredients which when fed to the animal is intended to supply its essential nutritional requirements. The feed must contain the proper nutrients in the proper ratios and in highly digestible and available forms. In addition, it must be palatable and attractable to the candidate shrimp species and meet appropriate water stability criteria. Feeding is the process of presentation of feed to the animal for consumption. Ideal feeding would provide each animal with the exact amount of feed it needs and can eat in the pond and at the proper time.

In the commercial shrimp aquaculture feed represents the single most important economic factor often contributing 45 to 55 % of the operating costs. The growing trend of intensified production further reiterates the significant role of feed as the level of intensification increases with increased use of quality feeds and thereby increasing the feed cost. In this context under feeding and over feeding has a direct bearing on FCR and thereby affecting the overall economics of shrimp farming. Aquatic animals like shrimp are most sensitive to the feed quality as compared to the terrestrial animals. The consistent production of high quality feeds is dependent on feed ingredient quality, feed processing technology and nutritional composition. Hence the quality of feed as well as the system for handling and delivery of feed and feeding management assumes much more significance in the context of cost effective production and sustainability of the shrimp farming.

2. Significance and current status of shrimp feed in Indian aquaculture:

The significant increase in shrimp aquaculture has been possible with the corresponding increase in the availability of formulated feeds. Shrimp feeds have been developed for sustainable and commercial culture of shrimps to meet the animal protein needs in developing countries (Smith, 2018). The principal cost in the manufacture of aqua feeds is that of raw materials; this could amount to as much as 80 percent, or more, of the manufacturing costs in commercial feed mills. Aqua feeds in India have evolved commercially in the late 90s for shrimp culture and then for fish during the last decade. In the brackishwater aquaculture sector majority of the feed used is scientifically formulated compounded feed produced by multinational / Indian companies. There are some small players with a capacity to produce 1 tonne per hour are entering the market.

Most of the corporate and big feed mills are having the state of the art facilities for the production of quality water stable shrimp feed pellets. The shrimp feed mills have facilities for coarse grinding, fine grinding, sieving, mixing and steam pelleting with three-stage pre conditioner combined with post-pellet conditioner is in vogue. Most of the shrimp feed produced in India uses a ring die pellet mill to produce a sinking compact pellet. In general, the shrimp feeds for tiger shrimp/Indian white shrimp consist of three grades of crumble (C1, C2 and C3) and three to four grades of pellets in the diameter of 1.8, 2.0 and 2.2 mm. However, there is a gradual shift in sizes of the pellet in the feed meant for pacific white shrimp, *Penaeus vannamei*. Now the feed pellet sizes available as 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mm are being used for feeding vannamei with one or two crumble sizes in the initial 30 days (Ambasankar et al., 2017). Though the production of feed pellets at sizes less than 1.8mm is a high energy consumption process, feed millers and farmers are of the view that smaller pellets are better and could able to meet the requirement of the current practice of white shrimp farming at higher densities than *P.monodon*. Attempts have been made to produce extruded shrimp feeds with reasonable success and few firms started producing the extruded shrimp feeds.

3. Feed Management

Feed represents the largest variable cost in shrimp aquaculture and is the single most factor influencing the growth of the shrimp as well as pond water quality and contributes directly for the improved profitability of shrimp aquaculture. Feed management can be defined as control and use of feed for aquaculture operation in such a manner that the utilization of feed is optimum with minimum wastage, negligible impact on environment, achieving best feed conversion ratio (FCR), better soil & water quality, maximum growth of shrimp and production (Anderson, 2020). Here it is to be kept in mind that a best feed can produce poor results if the feed management is poor. Alternatively, a moderate feed can produce best results under good feed management and hence feed management assumes considerable significance Feed management starts from the procurement and ends with the best use of the feed for a good harvest on the final day of shrimp culture and the BMPs in feed management required to be followed by farmers for successful shrimp culture are given below.

4. ***How to select a quality shrimp feed?***

A quality feed in the common parlance is that ability of the feed to give good growth and FCR without affecting the shrimp health and pond water quality (Johnson, 2019). Hence, the quality is comprised of the physical, nutritional and biological quality of the feed. The first and fore most point farmers looking up to select the feed is its physical quality.

4.1 Physical quality:

The feed pellets should be of uniform size and appearance, physical integrity and without any fine dust/powder. The feed should be fresh with good fishy odour and presence of too much or too less odor is not ideal. There should be any clumps and visible fungus. The physical integrity and nutrient leaching are of high importance in shrimp feed quality because of not only financial loss due to the loss of nutrient but also the implications it will have on environment. Farmers are evaluating the physical stability of the feed pellets to retain its physical integrity without nutrient leaching and disintegration. The feed should absorb minimal water so that it can become soft for ease of consumption.

4.2 Chemical quality:

The aqua feed to be selected should match the nutritional requirements of shrimp. Though most of the feed manufacturers are giving the nutritional composition the farmers should get verify the composition by analyzing its nutrient composition. The feed should be free from antibiotics and banned chemicals.

4.3 Biological quality:

The fed should be attractable and palatable to the shrimp and there should not be any problem in intake. . It should be highly digestible with negligible waste generation resulting in good growth with better FCR and should maintain the pond water quality within the optimal quality.

5. Feed storage

Feed storage is also an important component of fed management. On receipt of feed batch, all the feed bags should be weighed and examined for physical damage, opened or broken signs including the signs of repacking. The date of manufacture and the expiry should be ascertained and the points to be considered for storage are

1. The feed should be stored in a dry, cool well-ventilated area
2. Shrimp feed should be stacked to a maximum of 10 bags per stack
3. Direct storage from the floor should be avoided and preferably 1 to 2 feet away from the walls
4. Do not trample on the bags
5. Once opened it should be consumed at the earliest
6. Always use the policy of first in; first out so that there are no old stocks are cleared
7. Too much handling has to be avoided as it increases the fine dust in the feed bags
8. The storage area should be insect and rodent proof

6. **Feed management during first 30 days of culture:**

The feeding is proportional to the body mass in in all living animals including shrimp. However during the first 30 days of culture ascertaining the actual standing biomass is difficult. Hence the concept of blind feeding is practiced. This based on the assumption of approximate survival and bodyweight. During the first 30 days the concept of blind feeding is practiced. To start with approximately 100% of the body weight and at the end of thirty days it will be gradually reduced to 8-6%. However, for ease of understanding and use all the feed manufacturers are recommending a feeding guide based on their experience and assumptions. The total quantity of feed offered during the first 30 days are less than 10% of the total feed used in the culture and hence there will not be any problem during the blind feeding period even if the fed given is little more than the requirement.

During this period the feeding has to done along the dikes as the post larvae are having the tendency to move along the sides of the pond dikes. The fed used during this period will be in the form of crumbles and the chances of feed crumbles blown out of the pond due to the wind speed and also the feed has the chance for floating due to the lower density of the crumbles. Hence d it will be advisable to mix the feed with 10 to 15% of the pond water and then broadcast so that the feed will be broadcasted into the pond and facilities immediate sinking.

7. *Feeding from 30 days to the harvest:*

From the end of 30 days onwards feeding assumes much more significance and the actual requirement has to be ascertained by calculating the standing biomass as well as the average body weight of the shrimp. The sampling has to be carried out at 10 days interval and the biomass and average body weight has tom estimated to calculate the feed requirement at 10 days interval. By sampling 4 to 5 times the approximate survival and number of animals present and the average body weight are calculated and then the fed requirement per day is calculated as follows

$$Feed\ required\ per\ day\ (10\%) = \frac{ABW \times No.of\ animals}{1000} \times \frac{10}{100}\ kg$$

The calculated quantity is divided into four meals and fed four times a day. Though more number of feeding frequencies is advantageous, considering the practical difficulties a feeding frequency of 4 to 5 is advisable**.** The recommended feeding time is 6AM, 10AM, 2PM and 6PM and will depend on the temperature. If the morning temperature is too cold during winter period the feeding can be delayed by 30 -45 minutes. During the hot summer period the 2PM and 6PM feeding can be delayed by 1 hour

From 30^{th} day onwards Check tray monitoring is must. Preferably 4 Check trays are to be placed per hectare and feeding has to be adjusted based on check tray observation. To begin with 0.5% of the feed offered at a particular feeding time is calculated and kept in the check trays and observed at the end of 2hours and 30 times. As the culture increases the % of feed offered increases and it reaches a maximum of 1% when the shrimps reaches the harvest stage (25g). The check tray monitoring time has to be gradually decreased from 2 hours 30minutes during 30 to 60 days to 1hour 30 minutes at the end of the culture. The quantity of feed required for the next meal should necessarily be based on the check tray monitoring and the following guidelines are to be followed in deciding the feed requirement based on the check tray observation.

Quantity of leftover feed at the prescribed time	Change in feed quantity
No pellets remaining	Next meal can be increased by 5.0%
Fewer pellets to up to 5% of the feed remaining	No change and keep the same quantity for the next meal
5 to 10% of the feed remaining	5% decrease in quantity of the meal for the next time
10 to 20% of the feed remaining	10% decrease in quantity of the meal for the next time
20 to 30% of the feed remaining	20-30% decrease in quantity of the meal for the next time
More than 30% of feed remaining	Stop feeding for the next meal and start feeding again but decrease the quantity by 50% and increase to normal when the total feed is consumed

All the feed manufacturers use to give the feeding chart but farmers has to keep in mind that these are guidelines only and not the final one. Actual feeding will vary from pond to pond and so many factors are contributing for the feed intake y. Feed has to be adjusted on actual consumption basis and will vary based on growth, molting cycle, stocking density, natural productivity, soil and weather quality and prevailing atmospheric conditions. A farmer is the best judge as he knows the scenario in his pond better and the feeding has to be in line with check tray monitoring only.

Vannamei being amenable for higher stocking densities the feed requirement per time sometime increases beyond the manageable level of the feed boy and proper feeding is doubtful. The use of auto feeders is advantageous and the beneficial effect of auto feeders are not only reducing the labor requirement but helps in improving the growth, FCR and soil and water quality. A clear reduction of FCR up to a tune of 0.1 to 0.15 has been demonstrated through use of auto feeders. The increased feeding frequency and the constantly moving nature of vannamei resulted in the beneficial effect of feeding through auto feeders and one auto feeder is sufficient per pond with water spread area of up to 1 hectare. By following the above BMPs in feed management farmers can improve their profitability.

An understanding of chemical and biological conditions of pond soil and water through regular monitoring systems and adoption of efficient and careful feed management practices will lead to enhanced production of fish food organisms and thereby increase the growth and survival of fish.

8. Conclusion:

The quality of the feed will determine the final benefit provided to the farmer. However, **a** good-quality feed without proper feed and pond management practices will have no impact on a farm's profitability. Good-quality feed if properly processed through use of suitable feed ingredients with proven nutrition and researched feed formulation, combined with the correct feed management practices, will provide shrimp with a balanced nutrient profile, allowing them to thrive and grow and enabling shrimp farmers to see an increase in feed efficiency and a good economic return on their production thereby leading to sustainability.

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Chapter: 3

Climate Change and Aquaculture: The Need for Changes in Fish Nutrition and Aquafeeds

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1. Introduction

Climate change is a threat to all sectors, especially the food producing sector like aquaculture (Hamadan et al.,2015). Fish, being a poikilothermic animal is directly influenced by the changes in water temperature and therefore, increase or decrease in water temperature affects the growth and reproduction in fish. Similarly, the changing precipitation rate and fluctuations in seasonal precipitation, prolonged winter and summer periods and other such conditions alter the feed intake, and physiology of fishes. Proper understanding on the effect of these changes on feed intake, growth and physiology of fish can support the aqua-culturist to have a climate preparedness to reduce the detrimental effects that lower the production from farms. Hence, there should be a re-orientation in fish nutrition studies and feed formulations on the basis of changing environmental scenarios.

2. Impact of Climate Change

Aquaculture is one of the sectors which produces lower levels of greenhouse gases compared to many other food producing sectors and the main greenhouse gas from aquaculture is carbon dioxide and (Barange et al., 2018). The climate change effects vary depending on the region, climatic zone, culture systems and the species cultured (Adhikari et al., 2018; IPCC, 2018). For example, indoor and high-tech systems like RAS, Biofloc etc, where complete environmental control is there, will not be much affected by the changes in weather, whereas the semi-intensive, and intensive open water culture systems will be susceptible to these changes quickly. Hence, the culture systems of developing and under developed countries will be more threatened by climatic variations. The direct effect of climate change in aquaculture includes changes in basic and reproductive physiology of fish and shellfishes, metabolic shifting, change in feed intake and feeding behaviour etc whereas, the indirect effects include the non-availability of sufficient feed ingredients, ecosystem and trophic level changes, algal blooms, stock shifting, changes in productivity of aquatic systems etc (De Silva and Soto, 2009; Adhikari et al., 2018; Maulu et al., 2021). Therefore, it is well understood that climate change alters the optimum conditions required for the growth of fish and shellfish and the system will be subjected to different stressors anytime.

3. Stress and Aquatic Environment

Stress is the total expression of changes in physiological and biological systems due to intrinsic or extrinsic factors known as stressors. Fish living in an aquatic environment is always susceptible to biotic (Eg: pathogen, predators) and abiotic stressors (Eg: temperature, salinity, dissolved oxygen). These exposure gives certain response in them which is commonly known as General Adaptive Syndrome (GAS). Reduced feed intake is such kind of an important GAS, which is a distinctive feed back to the effect of stress (Bonga, 1997). During stressed condition, fish reduces the feed intake and thereby prolonged stress exposure results in slow growth. Sometimes, though fish maintain the feed intake, feed efficiency is found to be declined which results in reduced growth. These all changes are linked with endocrine growth stimulators. There are several factors influencing these endocrine stimulators like temperature, hypoxia, photoperiod, circadian rhythms etc.

4. Why Dietary Interventions?

Fish nutrition is a very important inward factor because feeding primarily depends on the energy needs of the fish, which are related to feeding behavior and feed consumption. This has the potential to have a lasting impact on the fish's appetite, which will then show up in its growth. In addition to being components of cellular biomass, nutrients are biological composites engaged in energy-producing biochemical activities. The two groups of nutrients that these have been divided into are macronutrients and micronutrients. Since they are the primary source of energy needed by organisms to survive, grow, and reproduce, macronutrients are needed in rather significant quantities. Conversely, micronutrients are required in smaller amounts, despite the fact that they have important roles in cellular functions.

Certainly, fish physiology and nutrition have been directly impacted by climate change, which may have negative effects on fish performance and health. The requirement of micro and macronutrients varies significantly based on the environmental changes. Changing climatic factors like temperature, pattern of precipitation, saline ingression, hypoxia etc leads to increased cortisol secretion in fish and thereby increasing the energy demand for maintaining normal physiology. This demands a higher requirement of macronutrients and several micronutrients. Generally, the high intensity cultivation of fishes in indoor and outdoor systems brings crowding and associated starvation in fish. Increase in temperature along with these factors severely affect the growth and reproductive performance of fish. Thus, a clear-cut understanding is required in the nutrient dynamics, changes in digestive physiology and metabolic shifting. Moreover, based on this understanding the diet for each species needs to be revised. Supplementation of several micronutrients are found to be effective in mitigating climate change related stress responses in fish (Fawole and Nazeemashahul, 2023). Reproductive performance of catfish can be enhanced by supplementing micronutrients like zinc, selenium and vitamin D in delayed monsoon conditions (Shamna et al., 2021). Therefore, a nutritional intervention-based mitigation strategy is essential to assure sustainable aquaculture and lessen the consequent harmful impact of this global warming on cultured fish.

5. Climate Change, Global Warming and Feed Intake in Fish

Temperature changes influence several biological functions in fish (Kasumyan and DÖving, 2003). Increase in temperature can enhance metabolism of fish and higher metabolic rate alters the physiological processes like respiratory rate, oxygen consumption and feed intake. Fishes shows an increased feed intake during higher metabolic rate. Voluntary food intake in fish generally increases with moderate temperature increments and the feed utilization and conversion efficiency varies with fish size. However, such changes are possible until the temperature level reaches to a certain extent. Feeding behaviour may decrease outside the optimal temperature range and it has been observed that fluctuations are observed in orexigenic and anorexigenic genes under extreme climatic condition which considerably affects the feed consumption (Assan et al., 2021). In general, the feed intake and temperature are having a bell-shaped relationship, the slight increase and decrease of temperature above the optimal level helps the fish to adapt to that situation and maintain feed intake (Fig 1). Once the animal cross thermal maxima or minima, it reaches to a stage in which appetite loss and termination of feeding are common. For example, Radhakrishnan et al. (2021) reported that decrease in temperature below 12°C resulted in complete appetite loss in carps. Thus, changes in water temperature results a change in basic mechanism in fish like feed intake and feed utilization. Hence, climate change has an influence on fish nutrition. Moreover, it has been observed that several food or food derived components can reduce stress in fish (Ciji and Akhtar, 2021) and act as feeding stimulant (Kinnera et al., 2023). Therefore, feeding functional additives or nutrients can enhance the stress mitigation and stimulate feed intake.

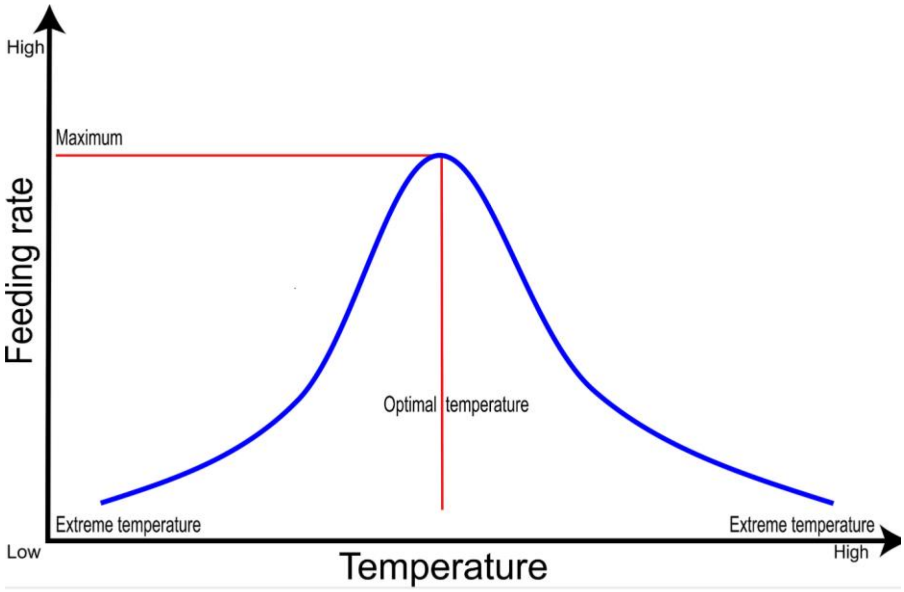

Fig 1: Relationship between feeding rate and temperature (Assan et al., 2021)

Another major output of climate change associated with temperature change is hypoxia. In order to conserve energy, fish exposed to hypoxic condition exhibits reduced feed intake (Remen et al., 2012). Magnoni et al. (2018) reported that hypoxia exposed rainbow trout showed a lower feed intake and energy and lipid content in body. However, several studies showed that supplementation of amino acids mitigate hypoxia related stress in fish.

6. Application of nutraceuticals

The quality of feed determines the nutritional effectiveness and utilization of a feed in fish and it depends on the attractiveness, digestibility, and stability. Only when the exact amount of energy and essential nutrients are present in the feed in sufficient amounts to support efficient feed intake, growth, health, and maintenance are considered as good feeds. The variation in fish feed components results in differences in response to feed intake, as well as in the taste and digestibility of the feed. This means that fish farmers need to pay attention to the quality of the feed they provide their fish because it affects how well the fish absorb it, how tasty it is, and how easily it can be digested. Likewise, a diet's composition serves as an extra nutritional because it affects hormones that control hunger, an element of a feed that requires careful consideration. Although there is not enough research on this topic, as noted by Bertucci and colleagues, a number of studies have shown that altering the macronutrient composition of the fish's diet significantly affects the expression and/or secretion of hormones that control appetite. Because changing food and/or environments affect fish growth and reproduction, it is crucial for aquaculturist to do application of stress mitigating compounds in diet. The use of immunoceuticals, antioxidants, and anti-stress chemicals is one practical way to do this. Immunostimulants have the capacity to stimulate fish defense mechanisms even under stressful situations, counteracting the negative effects that stressors have on fish (Ortuno et al., 2003; Shamna et al., 2020, 2021). Numerous nutritional supplements, including mannan oligosaccharides, propolis, astaxanthin, glucans, vitamin C, and tryptophan, have been studied for their potential to strengthen fish immunity and shield fish from stresses connected to severe temperatures. In pufferfish (*Takifugu obscurus*) kept at high temperatures, dietary astaxanthin supplementation enhanced growth performance, increased hepatic superoxide dismutase, catalase, and stimulated HSP70 (Cheng et al., 2018). In *L. rohita* fingerlings, L-tryptophan supplementation reduces heat stress, promotes development, and alters immunity (Akhtar et al., 2012; Kumar et al., 2014). It has been shown that when exposed to cold temperature stress, gilthead seabream fed a winter feed fortified with vitamin C, E, choline, taurine, phospholipid, and polyunsaturated fatty acids exhibit an enhanced immunological response (Tort et al., 2004; Schrama et al., 2007). According to the scientists, adding 100 mg of pyridoxine per kg of food could counteract the harmful effects of high temperatures and shield fish from temperatures as high as 33 ^{o}C in the water.

There is a lot of study being done on the application of antioxidants and anti-stress medications in fish, however on the context of climatic changes, it is a new and emerging area. For African catfish, *Clarias gariepinus*, under hyperthermia stress, the addition of vitamin C and iron nanoparticles at 6 g/kg and 4 g/kg, respectively, was found to be effective as stress-reducing agents (Gbadamosi, 2021). Supplementing vitamin C to hybrid grouper raised in acute low temperature stress caused their cortisol levels to drop as compared to the control group's greater concentration (Hao et al., 2017). The antioxidative properties of vitamin C may have a role in controlling the release of the stress hormone cortisol, which in turn may promote hepatic gluconeogenesis and elevate glucose synthesis in response to stress. Through cortisol-dependent acceleration of gluconeogenesis, cortisol is known to regulate glucose mobilization, and high blood cortisol levels are thought to be a sign of stress (Wendelaar Bonga, 1997). Hence, these studies indicating that supplementation of nutraceuticals in the diet can support the fish to cope up with the GAS associated with the high and low temperature stress.

7. Climate Change, Global Warming and Gut Microbiome

Changes in temperature and other related factors alters the gut microbiota including the presence of friendly microbes. During high temperature and anoxic conditions, microbial dysbiosis were observed and disruption of gut microbiota can pose potential threat to fish health. When water temperatures deviate from the control level, significant dysbiosis occurs in both the fecal and skin mucus microbiota of fish (Gosh et al., 2022). *Proteobacterium* is the predominant microbial group in the tract of fish followed by Bacteriodetes and Firmicutes (Rombout et al., 2011; Ghanbari et al., 2015). However, at high and low temperatures, *vibrio* and *Tenacibaculum* species were found, respectively. Study on warm water exposed *Salmo salar* showed an abundance of vibrio species (Neuman et al., 2016) and the *Gammaproteobacterium* in feaces indicates the abundance of facultative anaerobes in fish at high temperatures. This indicates that extreme change in climate results in multiplication of pathogenic bacteria that may affect the immunity of the host. Flavobacterium is found to be one of the major temperature specific genera that can be used as a biomarker for studying the effect of gut microbial variations. Therefore, temperature is found to be a key factor in maintaining gut friendly bacteria in fish (Gosh et al., 2022). Depending upon the climatic variations, environmental selection can result in specific gut microbial assembly. This can affect the microbial digestion in fish and the production of various metabolites like short chain fatty acids, vitamin B12 etc.

8. Maintaining gut health

According to recent studies, non-traditional foods can affect the gut microbiota. For instance, chitin-rich insect meals play a prebiotic role and boost immunity and gut health (Huyben et al. 2019). Common species found in groups fed insect meal include *Pseudomonas* sp. and *Lactobacillus* (Bruni et al. 2018). Apart from proteases, carbohydrases, and lipases, the digestive enzymes cellulase, chitinase, phosphatases, and others are produced by the fish gut microbiome and can improve digestibility (Ray et al. 2012; Wu et al. 2015). Hence, supplementation of acidifiers, prebiotics, probiotics, synbiotics and postbiotics can enhance the gut health and growth of friendly bacteria.

9. Climate Change, Global Warming and Digestive Physiology and Biochemistry

The changes in climate and associated temperature changes affects the digestive physiology and metabolic rate in fish depending on factors like duration of exposure, intensity and the quickness in temperature fluctuations (Volkoff and Rønnestad, 2020). Acute temperature variations cause drastic, mostly detrimental effect on fish physiology, whereas long term gradual exposure results in acclimation, altering digestive and metabolic enzyme activities. Understanding how fish react to changing environmental conditions is very critical and to respond to changes in the availability of natural food organism, fishes show adaptation by changing the digestive physiology and metabolic shifting. For example, the warm water fishes have a high affinity towards omega 6 fatty acids, while the coldwater fishes prefers omega 3 fatty acids for maintaining membrane fluidity at low temperature. However, the exposure to extreme temperature enhances the requirement of these nutrients and sudden low temperature exposure of warm water fishes and high temperature exposure of coldwater fishes due to climate change make them to adapt or switch from their normal preferences.

10. Ingredients, Formulations, Feed technology and Feeds

Eco-friendly feeds with lower carbon footprints are essential for sustainable aquaculture production. Fish stock shifting and agricultural cropping patterns may vary as a result of climate change. This could lead to a shortage of ingredients that are available in sufficient quantity and on schedule. One way to address the ingredient scarcity is to consider using seasonally appropriate and locally obtainable ingredients such as agri-by products, vegetable and fruit waste, leafmeal, seaweed meal etc. Furthermore, many of the traditional protein sources will be replaced by the increased production of insect meal and other invertebrate feeds. Reducing the impact of climate change on fish nutrition will also require a thorough understanding of nutritional needs and precision farming. Utilization of insect meals and methanotrophic bacteria meals which produce less greenhouse gases will be not only a better strategy to combat greenhouse gas emissions but also replacing fishmeal with quality sustainable ingredients. A lot of work has gone into finding environmentally fit substitutes for fishmeal, such as insect meal, single-cell proteins, algae, and other components derived from terrestrial plants. At the same time, research and documentation are required about the origin, hazardous chemicals present in the ingredients, anti-nutrients, and digestibility. Using organic wastes, such as fruit and vegetable wastes, will be one possible tactic to address the ingredient shortage (Atshaya et al., 2024). This will lessen the load on the ecosystem by breaking down large quantities of organic garbage produced. Another crucial tactic will be the use of non-edible seeds or their byproducts as aquafeed ingredients. The protein extracted from the non-edible seed cakes are found to be highly digestible and supports growth in fish (Shamna et al., 2015; 2018; Fawole et al., 2018; Jayant et al., 2021). The shifting crop pattern forces the researchers to include GM crops and its residues in aquafeed as an ingredient, especially the GM soybean meal. However, long term effect of same needs to studied thoroughly before introducing them in aquafeed.

Researchers and nutritionists focus on developing fish feeds that address changing environmental conditions and such feeds can support the fish to compensate extra maintenance energy requirements, lack of nutrient availability from changing natural feeds and survival under varying climatic scenarios. It is possible to modify the plant-based ingredient's nutritional quality through solid state fermentation, exogenous enzyme application, soaking, and protein extraction from components. The growth and feed efficiency of rohu fingerlings were found to be improved by solid state fermentation of leafmeal with *Chaetomium globosum*, according to Meshram et al. (2018). In a similar vein, Maiti et al. (2019) found that supplementing leafmeal-based fish diet with 0.1% exogenous enzymes (xylanse and cellulase) enhanced the fingerlings' ability to utilize nutrients. Fish feed utilization efficiency can also be improved by giving metabolic modifiers and using different feeding strategies. Changing the feeding rate and frequency depending on the fluctuations in temperature is also found effective. The system specific feeds are also found relevant in this context as the human interventions added up by climatic changes bringing ionic imbalances and saline ingressions in many parts of the country. It has been found that in such environment like inland saline waters, the nutritional requirements are varying for GIFT and Vannamei (Singha et al., 2021, Chuphal et al., 2021; Thirunavakkarusar et al., 2022; Garg et al., 2022; Paul et al., 2023). The studies from our lab showed that the supplementation of L-carnitine, and other metabolic modifiers enhances the osmoregulatory capacity, stress mitigation and growth in both GIFT and Vannamei reared in such harsh environment (Jana et al., 2022; Raghuvaran et al., 2022)

11. Selection of Fishes Based on its Genetic Potential

Another crucial tactic will be the identification of stocks and the selection of species based on factors such as feed conversion efficiency (FCE), hypoxia tolerance, temperature tolerance, or other relevant adaptations. The fish that have undergone genetic improvement are able to adapt to changes in their environment faster than their wild counterparts. Three spined sticklebacks that showed cold tolerance in just three generations are examples of how species selected based on temperature-associated SNPs (single nucleotide polymorphisms), immunological genes, or feed efficiency might lead to speedier adaptation (Barrett et al. 2011). Fish genetic selection can be based on traits such as heat tolerance, feed conversion efficiency, and thermal responsiveness for growth, which can produce quantifiable selection response (Ineno et al. 2008; Kavanagh et al. 2010).

12. Precision Nutrition

Feeding the necessary nutrients in the right amount at the right level can improve feed utilization and cut down on wastage. This may guarantee less organic matter and nitrogen being excreted into the system, potentially lowering pollution and eutrophication. The development of artificial intelligence (AI) and Internet of Things (IoT)-based automatic feeding devices that sense temperature changes and consequently detect changes in fish metabolism or physiological variation can reliably provide the animals with the necessary nutrients (Zhang et al. 2020). Accurate feeds can be achieved through the use of artificial intelligence and machine learning in aquafeed compositions. Similarly, sensors that identify alterations in the fish's physiology brought on by illness, malnutrition, or nutritional deficiencies can also be useful in feed management. The installation of self-feeders and sensor based automatic feeders revealed that fishes like rainbow trout could specifically differentiate diets having different amino acid content (Yamamoto et al., 2001). Fishes like sharp snout bream and Senegalese Sole exhibited a feeding behaviour in which macronutrients were selected according to the dietary requirements (Vivas et al., 2006; Rubio et al., 2009)

13. Conclusion

Understanding on the precise requirements of nutrients and supplying feeds with functional additives are a necessity in changing climatic scenario. Addition of nutraceuticals that can enhance the nutrient digestion and growth will be a better strategy to cope up the digestibility issues of unconventional ingredients. Additionally, development of system specific feeds and feed formulations for individual species will help in tackling the physiological changes in response to climatic conditions. Integrated aquaculture systems that successfully explore the many niches will be another crucial tactic.

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Chapter: 4

Innovative Feed Ingredients for Advancing Aquatic Sustainability

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1. Introduction

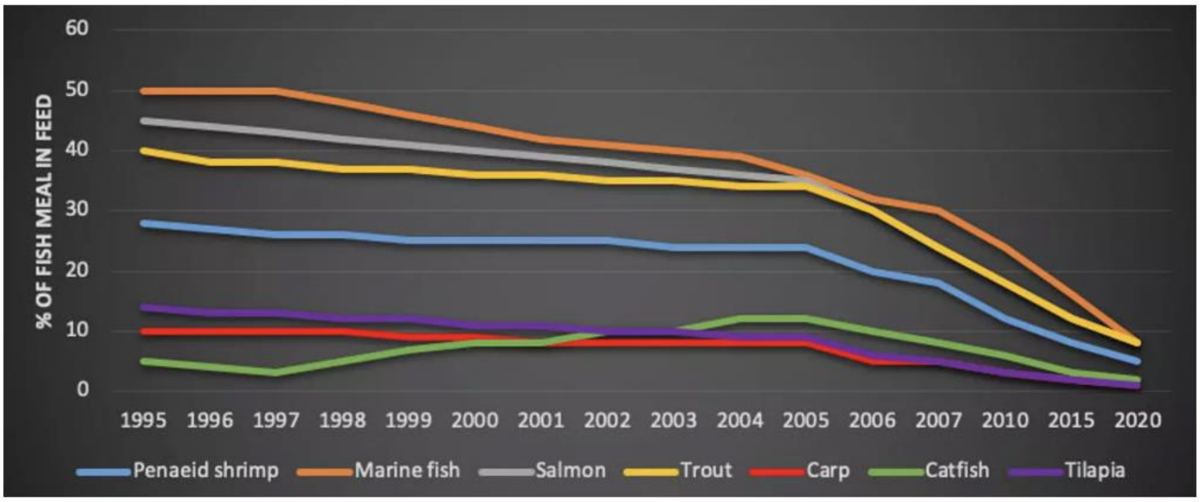
Aquaculture is the fastest-growing food production sector globally and is essential to meeting the projected 25–75% increase in nutrient-rich food supplies needed by 2050 (UN 2015; Hunter et al., 2017). The increasing middle class in Africa and Asia has boosted the demand for animal-sourced proteins (Goodman & Robison, 2013; Tschirley et al., 2015), leading to environmental concerns related to livestock production (Michalk et al., 2018). As a sustainable alternative, aquaculture helps reduce poverty and utilizes innovative culture systems (Filipski & Belton, 2018). Currently, aquaculture accounts for 52% of global fish production and provides 17% of total animal proteins, with a growth rate of approximately 5.8% annually from 2000 to 2018. However, about 70% of aquaculture production relies on aquafeeds, which constitute 40%-70% of expenses, underscoring the importance of efficient feed solutions. The aquafeed market, valued at USD 50.6 billion in 2020, is projected to reach USD 71.6 billion by 2025, driven by increased seafood consumption and aquaculture growth. Traditional aquafeeds heavily depend on fishmeal and fish oil (FMFO), raising sustainability concerns (Froehlich et al., 2018; Ghamkhar & Hicks, 2020). Projections indicate a 75% increase in aquafeed production by 2025 (Tacon & Metian, 2015). Addressing the sustainability of fed aquaculture is crucial, as feed costs can account for 40–80% of production expenses (Rana et al., 2009; Ayadi et al., 2012; Okomoda et al., 2020). Rising FMFO prices, expected to increase by up to 13% by 2030, further highlight the need for sustainable solutions (FAO, 2020; Tacon et al., 2022).

2. Fishmeal in Aquaculture

Fishmeal is a strategic raw material in aquaculture feeds, but the predicted increase in demand for fishmeal and fish oil will reduce their availability and drive up prices. This situation necessitates the use of alternative protein sources. Fishmeal, a nutrient-rich feed ingredient used primarily in animal diets and sometimes as a high-quality organic fertilizer, is generally made from wild-caught, small marine fish that are high in bones and oil and not suitable for direct human consumption. Adding fishmeal to animal diets enhances feed efficiency and growth by improving food palatability and nutrient uptake, digestion, and absorption. Its balanced amino acid composition works synergistically with other animal and vegetable proteins in the diet, promoting fast growth and reducing feeding costs.

Typically, feed accounts for 40–80% of aquaculture production costs (Rana et al., 2009; Ayadi et al., 2012; Okomoda et al., 2020). This cost continues to rise due to factors like cereal crop shortages, oil prices, global warming, and demand, disproportionately affecting small-scale producers and rural farmers (Rana et al., 2009). FMFO prices are projected to increase by up to 13% by 2030 due to global demand (FAO, 2020). The dependency on FMFO is recognized as the primary challenge to sustainability within the sector as aquaculture grows (Tacon et al., 2022). Due to the environmental impact and rising costs of forage fish-sourced meals, various raw materials have been adopted to reduce FMFO inclusion in aquafeeds. Currently, numerous plant-based products like soybean meal and rapeseed meal, as well as animal-based by-products like bone meal and poultry meal, are used as alternative protein sources. However, these alternatives have significant limitations and add pressure on land-based agriculture systems. Plant-based products often contain antinutritional elements and high fiber content, negatively impacting growth performance, health, and nutrient digestibility in aquaculture species (Okomoda et al., 2020). Additionally, they lack long-chain polyunsaturated fatty acids (PUFAs) and essential micronutrients crucial for the health and development of aquaculture species, particularly during larval and nursery stages (Malcorps et al., 2019).

Identifying sustainable, healthy, and cost-effective ingredients for aquafeed formulations is imperative. Finding alternative sources that can replace fish-derived ingredients and mitigate the disadvantages of vegetable protein sources, even at low inclusion rates, is a top priority (Cottrell et al., 2020). These ingredients must enhance growth, feed efficiency, and fish health while being environmentally, socially, and economically sustainable when mass-produced.



Percentage of fishmeal used in aquaculture feeds from 1995 to 2020

source: Tacon & Metian, 2008

3. **Advantages of Using Alternatives to Industrial Farm Animal Proteins**

- Gilthead sea bream fed with commercial and alternative diets that exclude fish and livestock byproducts showed better feed conversion ratios (FCR) and improved bioavailability of certain nutrients. Preliminary results also suggest these diets may reduce the fish's inflammatory response, though further trials are needed to confirm this. There was no difference in protein or phosphorus digestibility, both of which are critical for sustaining growth.
- For turbot and trout, the different diets did not affect growth rate or FCR. However, turbot fed alternative diets had a higher condition factor than those on conventional diets. Additionally, a consumer panel could not distinguish any differences in taste, odor, appearance, or texture between trout and turbot fed with different diets.
- Novel formulations based on sustainability and circular economy principles are viable options for these four fish species. The team is now examining the cost-effectiveness of these alternative diets and their potential impacts on fish health.

- Alternative proteins from sources such as insects, aquaculture, oilseed meals (e.g., soybean, canola, and sunflower), grains (e.g., wheat and corn), and legumes (e.g., beans and peas) offer several benefits:
- Contributing to a balanced diet
- Reducing unsustainable pressure on land, oceans, water, and energy
- Avoiding human infectious diseases transmitted by animals
- Utilizing farming lands to produce a variety of products
- Reducing animal wastes and greenhouse gases
- Meeting the food requirements of a growing human population is crucial. Insects, in particular, reproduce easily, convert feed into biomass efficiently, and can be reared on smaller surfaces. Among the nearly one million recognized insect species worldwide, at least 16 have been evaluated as alternative protein sources for aquaculture (Henry et al., 2015; Nogales-Mérida et al., 2019; Guerreiro et al., 2020). According to Alfiko et al. (2022), only eight of these species have shown promising results in scientific studies. These include:
    - Silkworm Bombyx mori (Ji et al., 2015; Nuswantoro & Rahardjo, 2018; Wu et al., 2021)
    - Black soldier fly Hermetia illucens (Katya et al., 2017; Dumas et al., 2018; Zarantoniello et al., 2019)
    - Housefly maggot and pupae Musca domestica (Emeka & Oscar, 2016; Kolawole & Ugwumba, 2018; Achionye-Nzeh & Ngwudo, 2021)
    - Mealworms, including yellow mealworm Tenebrio molitor and lesser mealworm Alphitobius diaperinus (Yi et al., 2013; Su et al., 2017; Rumbos et al., 2019; Jeong et al., 2020; Basto et al., 2021; Kurečka et al., 2021)
    - Crickets, including house cricket Acheta domesticus, banded cricket Gryllodes sigillatus, and Jamaican field cricket Gryllus assimilis (Vandeweyer et al., 2018; Nikoletta, 2019; Hessler Frelinckx, 2019; Józefiak et al., 2019; Tilami et al., 2020; Masson et al., 2020; Yue & Shen, 2021)
    - These insect species have high crude protein content ranging from 42–60% and are comparable to fishmeal and soybean meal in essential amino acids (Henry et al., 2015; Allegretti et al., 2017).

4. Alternative Ingredients to Substitute Fish Meal

- The complete or partial replacement of fishmeal with alternative protein sources has demonstrated equal or superior growth in animals compared to control fishmeal diets. For instance:
  - Rainbow trout (Oncorhynchus mykiss) fed with 25% yellow mealworm protein meal
  - Pacific white shrimp (Litopenaeus vannamei) fed with 20% microbial biomass
  - European sea bass (Dicentrarchus labrax) fed with 18% freeze-dried microalgae

4.1 Insects:

Insect-based feed materials are arguably the most important topic of interest in aquaculture nutrition (Barroso et al., 2014; Henry et al., 2015; Nogales-Mérida et al., 2019). Over the past two decades, research on insect meals as alternative sources for fishmeal and soybean meal has shown promising results (Nogales-Mérida et al., 2019; Xu et al., 2020; Alfiko et al., 2022). These studies have included meals made from various developmental stages of insects, such as larvae, pupae, and adults (Karthic et al., 2019; Sogari et al., 2019; Van Huis, 2020; Hawkey et al., 2021). Recently, the European Commission approved the inclusion of insects in the diets of aquatic organisms (Regulation 2017/893/EC, 2017). Insect-based feed resources are considered potential alternatives to expensive conventional ingredients not only due to their comparable nutritional components but also because of several other advantages (Barroso et al., 2014; Henry et al., 2015; Nogales-Mérida et al., 2019). These advantages include a reduced environmental impact in production and processing and the potential for converting waste to wealth, as insects thrive on waste and by-products with high conversion efficiency (Zarantoniello et al., 2018).

4.2 Single Cell Organisms (SCO)

Single-cell organisms (SCO), including bacteria, fungi, microalgae, and combinations like biofloc, have proven to be excellent substitutes for fishmeal and fish oil (FMFO) in terms of nutritional composition and feed effectiveness. Incorporating SCO in feed has led to improvements in growth performance, immunity, health, and quality of fish (Shah et al., 2018; Richard et al., 2021). SCO production is considered sustainable because they grow rapidly, use minimal fresh water, and do not require agricultural land. Additionally, they can be produced from non-food waste streams and aquaculture wastes (Viegas et al., 2021; Albrektsen et al., 2022).

Bacteria offer the advantage of rapid growth on organic substrates such as methane, methanol, syngas, carbon dioxide, hydrogen, and second-generation sugars (Matassa et al., 2020). Gas-based fermentation technology utilizes natural gas as a carbon and energy source to produce methanotrophic bacterial meal. With natural gas being plentiful and low-cost, protein production from natural gas is a viable large-scale alternative. Bacterial meal contains up to 80% crude protein (mean = 60%) and around 10% fat, making it comparable to fishmeal (Glencross et al., 2020; Albrektsen et al., 2022). Studies have shown that salmon and trout fed diets with up to 55% and 38% methanotrophic bacterial meal (e.g., Methylococcus capsulatus, Methylobacterium extorquens) either displayed increased growth performance and feed efficiency or experienced no adverse growth effects (Aas et al., 2006; Øverland et al., 2010; Hardy et al., 2018).

4.3 Microalgae

Microalgae have significant potential as a sustainable replacement for FMFO in aquafeeds. They can be cultivated using seawater or wastewater on arid, infertile land with minimal nutrients (Viegas et al., 2021; Ahmad), while achieving a net biomass production higher than any terrestrial plant or animal (Rizwan et al., 2018). Microalgae biomass can accumulate high levels of protein and lipids suitable for fish growth and development, with crude protein content typically ranging from 40−70% (Nagappan et al., 2021). Additionally, they contain value-added components such as carbohydrates, vitamins, antioxidants, probiotics, carotenoids, and amino acids that benefit fish health and quality (Chen et al., 2021). Despite their promise as an alternative to traditional aquafeeds, several challenges persist, including safety hazards from the ability of microalgae to adsorb and accumulate heavy metals (Pavithra et al., 2020).

4.4 Seaweed

Global seaweed (macroalgae) aquaculture production reached 32 million tons in 2018, representing 51% of global mariculture production and valued at over USD 11 billion (FAO, 2020). Currently, more than 99% of seaweed cultivation occurs in Asia, with a growing contribution from Africa (FAO, 2020). The vast majority (approximately 95%) of seaweed produced, such as Japanese kelp and Japanese wakame, is intended for human consumption (Ferdouse et al., 2018). Recently recognized for its bioremediation potential, seaweed offers a highly sustainable production option. When used sparingly as a functional feed supplement, seaweed can enhance immunity and improve the stress tolerance of commercially valuable fish and shrimp, among other health benefits (Yin et al., 2014; Niu et al., 2018; Øverland et al., 2019).

4.5 Algae-based protein:

Although algae have been incorporated into diets and research for years, their role as an alternative protein source is gaining momentum. Companies producing algae-based meat substitutes typically begin with microalgae (single-celled organisms), which undergo fermentation to develop a key ingredient. This ingredient is then combined with various flavorings to create the final edible product, utilizing different types of algae for specific purposes.Algae are rich in omega-3 fatty acids and essential vitamins. However, due to the early stage of development in microalgae-based foods and limited production, there are significant uncertainties surrounding the isolation and scalability of algae-based proteins.An important challenge for algae as an alternative protein source lies in its distinctive smell, color, and taste, potentially limiting acceptance among the broader population. Continued research and investment are expected to clarify the potential benefits and drawbacks of scaling up algae-based protein production.

5. Qualitative Feasibility Assessment of Alternative Protein Sources

Alternative protein sources from plants include soybean meal, cottonseed meal, corn gluten meal, rapeseed meal, canola meal, peanut meal, guar meal, sunflower meal, moringa oleifera, rice protein concentrate, and brewer’s spent grains & spent yeast. Each of these sources has unique nutritional profiles, benefits, and limitations:

5.1 Soybean Meal (SBM):

Soybean meal (SBM) stands out as a secure, stable source of amino acids with high protein content and affordability among plant protein sources (Meng et al., 2020; Pervin et al., 2020). Numerous studies have demonstrated that SBM can effectively replace fish meal (FM) in the diets of various fish species (Nyirenda et al., 2000; Kalla et al., 2003). However, the effects of substituting SBM for FM protein on fish growth, physiological state, and feeding habits vary across species (Zhou et al., 2018). Generally, omnivorous and herbivorous fish species utilize SBM more efficiently than carnivorous species. Growth and physiological parameters remain largely unaffected by the full or partial replacement of FM with SBM in several fish species (Liu et al., 2021). Siddique et al. (2014) reported that replacing 15% of FM protein with SBM did not significantly impact the growth of H. fossilis fry, suggesting potential for higher levels of SBM inclusion. Similarly, Pervin et al. (2020) found that replacing 75% of FM with SBM had no significant effects on Oreochromis niloticus. Mohammadinafchi et al. (2014) observed no discernible differences in Mesopotamichthys sharpeyi when FM was completely replaced with SBM. However, the effects of SBM substitution on the physiology and growth performance of stinging catfish require further investigation, as documented research is limited.

Despite its advantages, SBM contains higher levels of anti-nutrients and lower methionine content, which restricts its use in fish feed (Ollie et al., 1994). Nevertheless, SBM remains a highly accessible and cost-effective substitute for expensive fish meal in aquaculture. Current research aims to determine how varying levels of SBM replacement for FM protein affect the growth, feed efficiency, and overall health of stinging catfish.

5.2 Cottonseed Meal:

Due to its cost-effectiveness and availability in countries such as the USA, China, India, and Egypt, cottonseed meal (CSM) has been studied as a potential substitute for both fish meal and soybean meal in aquafeeds. However, the protein content of CSM varies widely, ranging from 23% to 53% depending on the processing methods used (Mbahinzireki et al., 2001; Yue and Zhou, 2008). Research has explored the incorporation of CSM in various fish species, including Oncorhynchus mykiss (Lee et al., 2006), Sarotherodon mossambicus (Jackson et al., 1982), Oreochromis niloticus (Yue and Zhou, 2008), and Ictalurus punctatus (Robinson and Tiersch, 1995).These studies generally indicate that high levels of CSM inclusion can lead to growth reduction, whereas more favorable outcomes are observed at lower inclusion levels. Limitations to CSM incorporation in aquafeeds include amino acid imbalances, digestibility issues, and the presence of anti-nutritional factors (ANFs) like gossypol. These factors hinder nutrient utilization, thereby impacting growth, feed efficiency, and overall nutrient absorption (Francis et al., 2001; Li and Robinson, 2006).

5.3 Corn Gluten Meal:

Corn gluten meal (CGM) has been noted for its lower levels of anti-nutritional factors compared to several other plant-based protein sources (Hernandez et al., 2021), but it lacks sufficient levels of the essential amino acids lysine and arginine (NRC, 2011). While some studies have shown success in replacing fish meal with CGM in fish feeds (Bu et al., 2018; Kikuchi, 1999; Pereira and Oliva-Teles, 2003; Men et al., 2014), there is limited research on the potential for using CGM as a fish meal substitute in feeds for farmed mollusks.

5.4 Canola Meal

Canola meal, a prevalent protein source in animal diets, boasts a higher fiber content of 12% compared to soybean meal's 7%. It offers a protein content ranging from 32% to 45% of dry matter, which is comparable to soybean meal. Canola meal provides a well-balanced composition of amino acids and is known for its consistent supply and cost-effectiveness. Additionally, it supplies minerals, vitamins, and other essential microelements.

5.4 Rapessed Meal

Good protein content: 32 to 45% of dry matter next to soybean meal. Comparatively balanced amino acid content. Low cost and constant supply. Provides vitamins, minerals, and other microelements. Lower lysine value than Soybean Meal (SBM) but rich in sulfur amino acids

5.4.1 Anti-Nutritional Factors (ANFs) in RSM:

Include tannins, phytic acid, sinapine, erucic acid, glucosinolates, and indigestible carbohydrates

Adverse effects on fish growth and health due to:

- Low palatability
- Poor digestibility
- Decrease in feed utilization

5.4.2 Functional Bioactive Peptides:

- Released during microbial and enzymatic denaturation of rapeseed protein
- Perform specific biological activities including:
- Antihypertensive
- Immunomodulatory
- Antimicrobial

5.5 Peanut Meal

Pigeon pea (Cajanus cajan L.) is widely cultivated and serves as a cost-effective source of protein and energy in many third-world countries, particularly in India. While commonly consumed as dhal, pigeon pea is nutrient-dense but contains antinutritional factors that can be managed through various processing methods. This study explores the potential use of pigeon pea leaves as a substitute for DORB. The proximate analysis shows a moisture content of 4.80%, with the remaining 95.20% as dry matter. Within the dry matter, it contains 19.97% crude protein, 3.23% ether extract (fat), 16.55% crude fiber, and 7.65% ash. Non-fibrous carbohydrates (NFE) make up the majority of the dry matter at 52.61%. These findings provide a comprehensive overview of the nutritional composition of the sample.

5.6 Sunflower Meal

Rich in protein but limited by high fiber and phenolic compounds. Increased sunflower seed production for oil can yield more meal, potentially replacing costly soybean meal. Sunflower seeds grow worldwide due to their adaptability to various climates and soils. Sunflower seed meal (SFSM), a by-product of oil extraction for human consumption, varies in quality due to processing methods and seed types. Efficient methods can produce SFSM with 42-46% protein and 8-14% cellulose. De-hulling and oil extraction processes influence protein content (29-45%) inversely related to fiber (14-32%).

5.7 Moringa oleifera

High mineral content, though ANFs reduce palatability. Fresh Moringa oleifera leaves, known as 'drumstick', are highly nutritious supplements for plant-eating fish like tilapia, barbs, and fancy carps. They are rich in protein, lipids, vitamins, and minerals, making them valuable in aquaculture feeds. Processing methods such as drying, soaking, and grinding help reduce anti-nutritional factors, which are generally low in Moringa leaves except for saponins and phenols. Moringa leaves provide essential amino acids like methionine, cystine, and tryptophan, promoting fish growth and health. Studies show that Moringa leaves can partially replace conventional diets without compromising growth performance in species like Nile tilapia, common carp, and African catfish. However, caution is advised with high inclusion levels of Moringa leaf meals due to potential costliness and the risk of compromised growth and health.

5.8 Rice Protein Concentrate

High protein but deficient in lysine. . Rice bran is also a good source of B vitamins, vitamin E, and minerals such as magnesium, phosphorus, and manganese. Furthermore, it is an excellent source of dietary fiber, which is beneficial for digestive health. Additionally, rice bran contains various antioxidants, including tocopherols, tocotrienols, and oryzanol. The defatting process primarily affects the lipid content, as would be expected. However, it can also impact the levels of other components, such as phenolics.

6. Conclusion

To effectively incorporate plant proteins into aquafeed, several challenges must be addressed, including optimizing their nutritional content and ensuring technical feasibility. Ongoing research and innovation in alternative protein sources will be pivotal for promoting sustainable growth in aquaculture. Marine shrimp, fish, and anadromous salmon are major consumers of fish meal, relying on its high protein content for metabolic functions (Tacon et al., 2011; Tacon and Metian, 2015). Similarly, anadromous salmon, marine fish, and trout depend heavily on fish oil for essential polyunsaturated fatty acids such as EPA and DHA (Torstensen et al., 2005). However, the inclusion rates of fish oil vary significantly among species, with herbivorous and omnivorous freshwater fish requiring less compared to carnivorous species (FAO, 2021). Plant-based substitutes often have lower digestibility and less favorable amino acid profiles, and they may contain antinutritional factors that can impact growth (Gatlin et al., 2007). Despite ongoing research into alternatives like insects, which offer nutritional benefits, challenges remain concerning costs, processing efficiency, and ensuring consistent quality (Makkar et al., 2014). Addressing these challenges is critical for advancing sustainable aquaculture practices, reducing environmental impact, and enhancing resource efficiency.

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Chapter: 5

Algal Feed Formulations

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1. Introduction

Global fish production was estimated to have reached 204 million tonnes in 2025. Of these, 140 million tonnes came from aquaculture production. Among these, 182 million tonnes were used for human consumption. The remaining 22 million tonnes were destined for non-food uses, mainly to produce fishmeal and fish oil. The global farmed shrimp market continues to grow faster than other aquaculture species. From April to September 2019, Indian shrimp production (90% *Penaeus vannamei*) increased to approximately 700 000 tonnes (FAO, 2020). Feed is a major input in aquaculture system which supply all essential nutrients (protein, carbohydrate, lipid, vitamins and minerals) for regulating growth, physiological process and resistance against diseases (Bhavan *et al.,* 2011). Fishmeal is the most important ingredient of balanced feed formulas used in aquaculture. However, the rapidly growing aquaculture industry using fishmeal as a major protein source in compounded feed has strongly contributed to increased demand and prices for this product. Concern has been expressed that this may lead to over-fishing.

Also, it may not be ethically correct to harvest fish for aquaculture feed which could be used directly as food for humans (Nayler *et al.,* 2009; Tacon and Metian, 2009) Fish meal and Fish oil are especially targeted for use in protein-rich, energy-dense feeds designed for high value marine finfish and shellfish. Among the many plant ingredients tested in fish diets, microalgae have garnered perhaps the most attention because of their widespread availability, competitive prices, and generally favorable nutrient composition. Microalgae possess high protein contents and amino acid profiles comparable to those of other reference food proteins (Becker, 2007). Apart from the high protein content, microalgae have been receiving attention as an animal food source due to its rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Belay *et al.,* 1996; James *et al.,* 2006). In addition to the high nutritional value, microalgae serve as an effective immune modulator in fish (Watanuki *et al.,* 2006) and may have the potential for use as an antimicrobial agent in aqua feed (Pradhan *et al.,* 2012). The use of microalgae has been shown to enhance the innate immunity and increase the resistance against pathogenic infection in shrimp (Lin *et al.,* 2010). Therefore, considerable research has been done investigating the use of alternative ingredients to replace fish meal in feeds formulated for shrimp. The white leg shrimp *Penaeus vannamei* is commercially important species and has become the main farmed species in India. However, unfortunately, the emergence of diseases is a major threat and causes huge economic loss in prawn farming industry. To overcome this, developing the alternate feed ingredients which can provide higher resistance against pathogens is need of the hour. In this context, the present paper reveals the effects of replacing fishmeal with microalgae meal on growth, survival, body composition, dietary antioxidant capacity, metabolic enzymes level, innate immune responses and disease resistance of white leg shrimp *Penaeus vannamei* against shrimp pathogen *Vibriyo harveyi*.

2. Methodology

For isolation and purification of the algal strains, agar plating and serial dilution techniques were followed (Robert, 2005). The mixed cultures contained of different types of microalgae were diluted in 1/10 dilution series with sterile Walne medium (Walne, 1970). The individual colonies were isolated and inoculated in both liquid and agar slants of Walne medium, incubated at ambient temperature under light with 12:12 h light dark cycle. Algal stock cultures maintained separately in 250ml, 500ml, 1 and 2 litres conical flasks containing 20-23°C temperature, 5000 lux light intensity and fertilized with Conway's medium and Zorrouk's medium. At the stage of stationary phase microalgae culture were harvested by centrifugation at 3000rpm for 10 min and washed using distilled water. The biomass was dried at 60°C and stored in vacuum desiccator. The extracts were bought from exclusive solvents primarily based on the polarity (polar: Methanol, ethanol Intermediate: Ethyl acetate, Non polar: Hexane). 1mg/ml of the microalgae powder used to be taken for secondary metabolites extraction and soaked in different solvents and stored in room temperature for 24 hours respectively. After the extraction, the solvent was filtered through Whatman No. l filter paper. The crude was used for phytochemical analysis (Sanjeet *et al*., 2010), antioxidant (DPPH- Brand-Williams *et al.,* 1995, Hydroxyl Radical Scavenging Activity- Halliwell *et al.* 1987, Super oxide radical scavenging activity Ruch *et al.,* 1989) and antibacterial activity (Bashir *et al*., 2018). Analyses of proximate composition of microalgae was done according to the standard procedure of Price (1965) for protein, Dubois *et al.* (1956) for carbohydrate, Folch *et al.*, (1961) for lipid. The optimization of various culture conditions such as culture medium (Conway's, BG11, Bold Basal medium, f/2, Zorrouk), temperature (23ºC, 26ºC, 29ºC, 32ºC, 35ºC), pH (6, 7, 8, 9, 10), photoperiod (PP) (24:0, 18:06, 12:12, 20:04 and 6:18 h light:dark) and photosynthetic photon flux intensity (PPFI) (50, 100, 150, 200 and 250 μmol m^{-2} s^{-1}) on the production of growth, biomass and pigment (chlorophyll 'a' and phycocyanin) production of *Arthrospira platensis* were made. The growth, biomass, and pigments were assessed once in three days for 15 days. The estimation of chlorophylls 'a', and phycocyanin was carried out as per the method of (Lichtenthaler 1987 and Siegelman and Kycia 1978).

The feed ingredients such as fish meal, soybean meal, rice bran, tapioca, groundnut oil cake, egg albumin was purchased from the local store. Cod liver oil and vitamin B complex (Pfizer Ltd., Mumbai, India) were purchased from local pharmacies. All feed ingredients were ground separately using a micro pulvelizer and sieved through a 60μm mesh. The powered and sieved feed ingredients were weighed out and mixed thoroughly in 5 different ratios for preparing 5 different diets (1 control with fishmeal diet and 4 diets containing microalgae *A. platensis* at various levels such as 25%, 50%, 75% & 100%). The blends were cooked in a pressure cooker for 15min at 110ºC and were allowed to cool at room temperature. The cooked blends were mixed with vitamin (1g), cod liver oil (4ml) and egg albumin (5ml) and blending well until the mixture achieve a dough consistency. A manual pelletizer fixed with 3mm diameter and the pellets were collected in trays. Then the feeds were dried in a hot air oven until the moisture content was reduced below 10%. The dried feed pellet was visually examined for their physical appearance, colour, fragrance. The proximate composition such as protein, carbohydrate, lipid, moisture and ash were analysed according to the standard procedures of (AOAC 1995). The feed leaching was analysed by the method of Muralisankar *et al.* (2017).

In this study 5 groups of *P. vannamei* (PL-12) range from 0.9 to 1.7 cm in length and 0.06 to 0.12 g in weight were assigned in triplicate for 90 days. Each group contain 30PL×3=90PL, totally 90PL×5=450PL were maintained in separate for feeding experiment. The experimental group were fed with their respective diets, twice daily at 6am and 6pm. The daily feed ratio was adjusted to 10% of the body weight throughout the feeding period. The unfed feed, faeces and other metabolic wastes were removed on daily basis while renewing rearing water. The growth and nutritional indices such as survival, weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio were calculated by the standard method (Tekinay and Davies, 2001; Zhou *et al.,* 2007). Every 30^{th} day shrimp were sacrificed for analysing the proximate composition. The content of total protein in the ethanol precipitated sample was estimated following the method of Lowry *et al.* (1951). Total carbohydrate present in TCA extracted sample were estimated by the method of Dubois (1956). Total lipid was extracted with chloroform-methanol mixture following the method of Folch *et al.* (1957) and was estimated according to Barnes and Black-stock (1973). Amino acid was extracted using sodium tungstate and H_2SO_4 and analysed by the method of Moore and Stein (1948). The pre-weighed wet tissue sample were dried at 40°C in hot air oven until reached a constant weight to measure the moisture content. The dried tissue sample subjected to 600°C for 4 h in a muffle furnace to measure the ash content following the standard method (APHA 2005). Activities of digestive enzymes such as, protease, amylase and lipase were assayed on initial and every 30^{th} day of feeding period. In these test in each experiment, shrimp were sacrificed and the whole flesh except eye stalk, appendages and exoskeleton was homogenised in ice cold water and centrifuge 10,000rpm under 4°C for 20min. the supernatant was used as crude enzyme source. The total protease activity was determined by the casein- hydrolysis method described by Furne *et al.* (2005). Amylase activity was determined by the starch-hydrolysis method (Bernfeld, 1955). Lipase activity was determined following the method of Furne *et al.* (2005) by degrading triacylglycerol to free fatty acid. Estimation of Vitamin- C and E (non-enzymatic antioxidant) was measured according to the method of Roe and Kuether (1943). Estimation of enzymatic antioxidant activity SOD activity was measured using pyrogallol (10mM) autoxidation in Tris-buffer (50Mm, pH 7) according to the method described by Marklund and Marklund (1974). CAT activity was measured using H_2O_2 as the substrate in phosphate buffer (Sinha 1972). Lipid peroxidation was measured by estimating the formation of thiobarbituric acid reactive substrate (Ohkawa *et al.,* 1979). At the end of the experiment three shrimp randomly selected for haemolymph collection and histological investigation was made by the method of Culling (1983). After feeding trail challenging test was done according to the method of Harikrishnan *et al*. (2012).

3. Inference

Totally 25 species of microalgae were selected from different groups such as Chlorophyceae, Cyanophyceae and Bacilariophyceae for preliminary screening. Among these, 12 species of microalgae (*Arthrospira platensis, Tetraselmis suecica, Nanochloropsis oculata, Dunaliella salina, Picochloroum maculatum, Isochrysis galbana, Cosmarium botrytis, Anabena* sp*., Nostoc* sp*., Chlorococum* sp*., Ochrospaera verrucosa* and *Chlorella marina)* which contain 30% of protein were selected for further screening. Among the 12 species, maximum phyto-constituents (alkaloids, flavonoids, terpenoids, saponin, phenols, tannins, glycosides) were noticed in methanolic extract of *A. platensis*. Based on the phytochemical analysis, we have selected methanolic extract of microalgae for scavenging effect of DPPH, super Oxide Radicals, hydroxylradical assay. The highest antioxidant activity (73.23%) and antibacterial activity was noticed in *A. platensis* (11mm) and *T. suecica* (10 mm). Based on the proximate composition, phytochemical analysis, antioxidant and antimicrobial activity *A. platensis* was selected for further study.

Various culture conditions such as culture medium (Conway's, BG11, Bold Basal Medium, F/2, Zorrouk), temperature (23ºC, 26ºC, 29ºC, 32ºC, 35ºC), pH (6, 7, 8, 9, 10), photoperiod (PP) (24:0, 18:06, 12:12, 20:04 and 6:18 h Light:Dark) and photosynthetic photon flux intensity (PPFI) (50, 100, 150, 200 and 250 μmol m^{-2} s^{-1}) on the growth, biomass and pigment (chlorophyll 'a' and phycocyanin) production were optimized for *A. platensis* at laboratory scale. The growth, biomass, and pigments were assessed once in three days for 15 days. Influence of medium on the highest algal growth (2.93), biomass (0.248 μg/g) pigment (chlorophyll 'a' 4.37μg/ mL & phycocyanin 0.71μg/ mL) were achieved at Zorrouk's medium. The highest values for growth (1.16), biomass (0.51 µg/g), pigment (chlorophyll 'a' 2.98µg/mL and phycocyanin 0.38 µg/ mL) were noticed in 23°C. The pigments yield and growth of *A. platensis* cells were assessed in relation to the diverse pH conditions. The alga was found to survive in all pH conditions (6, 7, 8, 9 and 10) when tested using Zorrouk's medium. The maximum growth (2.59), biomass (0.204 µg/g) and pigment (chlorophyll 'a' 2.96 µg /mL &0.39 µg/mL) were recorded at the pH 9. Among the five photoperiods tested, the significant results were found at 12:12 h L: D photoperiod (under the culture conditions of 9 pH, 23 ∘C of temperature, and Zorrouk's medium). The highest values of growth (0.58), biomass (0.50 µg/g), pigment (chlorophyll 'a' 1.91 µg/mL and phycocyanin 0.70 µg/ mL) were noticed. The effect of light intensity on the growth and biomass accumulation showed significant outcome in 250 µmol m^{-2} s^{-1} of PPFI and minimum was found in 50 µ µmol m^{-2} $s^{-1}$of PPFI (under the culture conditions of zorrouk's medium, 9 pH, 23 ∘C of temperature, and 12:12 h L:D of photoperiod). The highest values of growth (0.696), biomass (0.70 µg/g), pigment (chlorophyll 'a' 1.99 µg/mL and phycocyanin 0.708 µg/ mL) were noticed in 250 µmol m^{-2} s^{-1}. Optimized condition for *A. platensis* growth was found in Zarrouk medium, pH-9, Temperature-23ºC, Photoperiod -12L: 12D hr and Light intensity- 250 µmol m^{-2} s^{-1}. Based on the positive result obtained, the mass culture of *A. platensis* was made and analyses of biochemical profile of mass cultured *A. platensis* was done in appreciable level (Protein - 59.36%; Carbohydrate - 9.14%; Lipid - 6.02%; Crude fiber - 1.0%; Moisture - 7.15%; Total ash - 8.294%). Recorded essential amino acids in *A. platensis* were Histidine (0.2291g), Valine (0.4011g), Methionine (0.1832g), Iso-leucine (0.1382g), Phenylanine (0.1192g) and Leucine (0.3082g), Lysine (0.1293g), Threonine (0.1833g), Proline (0.1382g) and Tryptophan (0.208g). Seven fatty acids were reported in *A. platensis* Palmic acid (0.4933g), margaric acid (0.1193g), stearic acid (0.3082g), Oleic acid (0.308g), linolenic acid (0.3382g), alpha linolenic acid (0.0793g) and morotic acid (0.0182g).

The proximate constituents such as total protein, carbohydrate, lipid, moisture and ash contents were analyzed in formulated control and experimental feeds. The control feed showed higher percentage of Protein (42.69 %), Carbohydrate (28.57%) and Lipid (8.69 %). Moisture was higher (4.31%) in 25% of feed. The ash was higher (11.94%) in 100% of feed. The leaching percentage of experimental diets was ranged from 18.85% to 19.05%. 50% showed lowest leaching rate (18.30%) followed by 25% (18.89%), 75%

(18.85%) & 100% (19.05%) when compared to control feed. The maximum amino acid was observed in 50% *A. platensis* incorporated feed when compared to other feeds. (Histidine-0.209g, Valine- 0.309g, Methionine- 0.183g, Leucine- 0.219g, Threonine- 0.209g, Lysine- 0.214g). The maximum fatty acids were noticed in 50% feed (Palmic acid 16:0- 0.283g, Margaric acid 17:0- 0.278g, Stearic acid 18:0- 0.908g, Oleic acid 18:1- 0.708g, Alpha linolenic acid 18:3- 0.658g, Morotic acid 18:4- 0.908g, DHA- 0.362g and EPA- 0.172g).

The larval rearing experiment on *P. vannamei* fed with replacement of fish meal with 50% *A. platensis* showed higher growth (Length - 12.9cm & weight - 12.09 gm) and survival (97%) compared to control feed fed animal (Length - 12.01cm & weight - 11.98g and survival - 94%). The proximate composition of shrimp fed with replacement of fish meal with 50% *A. platensis* (Initial day- 26.43±0.52, 30th day- 38.53±1.6, 60th day- 69.28±1.83, 90th day- 72.46±1.8) showed high protein when compared to 25%, 75% and 100% *A. platensis* incorporated feed fed animals. Experimental study revealed that various concentration of *A. platensis* treated groups were showed no significant changes on THC (Control- 6.5x10^{7}/mL, 25%- 8.5x10^{7}/mL, 50%- 9.0x10^{7}/mL, 75%- 8.0x10^{7}/mL and 100%- 9.5x10^{7}/mL). Essential amino acids were observed in *P. vannamei* fed on 50% *A. platensis* experimental diets showed high histidine (0.219g), iso-leucine (0.314g), phenylalanine (0.221g) and proline (0.209g). Seven fatty acids were observed in *P. vannamei* fed on 50% *A. platensis* experimental diet showed high margaric acid (1.083g), oleic acid (2.834g), linolenic acid (3.558g) and alpha linolenic acid (3.011g). The vitamin-E & C in Hepatopancreas content were found higher in *P. vannamei* fed with 50% Vitamin-c (Initial - 7.06 µmol/mg of protein, 30th day- 9.68 µmol/mg of protein, 60th day- 10.72 µmol/mg of protein, 90th day- 13.77 µmol/mg of protein) Vitamin- E (Initial - 4.24 µmol/mg of protein, 30th day- 7.97 µmol/mg of protein, 60th day- 9.56 µmol/mg of protein, 90th day-12.89 µmol/mg of protein) followed by 75%, 25% and 100% when compared to control group. SOD activity of muscle Control (Initial day-2.3±0.12, 30th day- 6.8±0.07, 60th day- 8.54±0.32, 90th day- 11.3±0.3 µmol/mg of protein), 25% (Initial day- 2.3±0.12, 30th day- 6.35±0.32, 60th day- 8.42±0.09, 90th day- 10.72±0.3 µmol/mg of protein), 50% (Initial day-2.3±0.12, 30th day- 7.06±0.14, 60th day- 8.72±0.48, 90th day- 11.51±0.3 µmol/mg of protein), 75% (Initial day-2.3±0.12, 30th day- 6.85±0.09, 60th day- 8.49±0.09, 90th day- 11.43±0.2 µmol/mg of protein) and 100% (Initial day-2.3±0.12, 30th day- 6.8±0.07, 60th day- 8.54±0.09, 90th day- 11.3±0.2). Hepatopancreas Control (Initial day-13±0.3, 30^{th} day- 16.22±0.09, 60^{th} day- 22.61±0.09, 90^{th} day- 29.73±0.07 µmol/mg of protein, 25% (Initial day-13±0.3, 30^{th} day- 15.9±0.25, 60^{th} day- 21.87±0.32, 90^{th} day- 29.25±0.32 µmol/mg of protein), 50% (Initial day-13±0.3, 30^{th} day- 16.52±0.7, 60^{th} day- 22.79±0.9, 90^{th} day- 29.63±0.3 µmol/mg of protein), 75% (Initial day-13±0.3, 30^{th} day- 16.38±0.08, 60^{th} day- 21.69±0.09, 90^{th} day- 28.82±0.0.05 µmol/mg of protein) and 100% (Initial day-13±0.3, 30^{th} day- 16.24±0.4, 60^{th} day- 22.18±0.9, 90^{th} day- 28.93±0.2 µmol/mg of protein) incorporated feed fed group showed no significant variation among control and experimental group. Catalase activity of experiment feed fed group no significant difference was observed on 50% & 25%. 75% and 100%. *A. platensis* incorporated feed fed group showed less catalase activity when compared with control. LPO activity in muscle and Hepatopancreas of 50% (Initial day- 0.91±0.14, 30^{th} day- 1.17±0.1, 60^{th} day- 1.96±0.41, 90^{th} day- 2.13± 0.42 and Initial day- 1.64±0.06, 30^{th} day- 2.32±0.08, 60^{th} day- 4.63±0.51, 90^{th} day-6.8±0.320.06 µmol/mg of protein) incorporated feed fed group showed no significant difference when compared with control (Initial day- 0.91±0.14, 30^{th} day- 1.17±0.1, 60^{th} day- 1,96±0.41, 90^{th} day- 2.13±0.4 and Initial day- 1.64±0.06, 30^{th} day- 2.32±0.08, 60^{th} day- 4.63±0.51, 90^{th} day-6.8±0.32 µmol/mg of protein) group. Hepatopancrease of 75% (Initial day- 1.64±0.06, 30^{th} day- 2.54±0.04, 60^{th} day- 4.78±0.07, 90^{th} day- 6.92±0.09 µmol/mg of protein) and 100% (Initial day- 1.64±0.06, 30^{th} day- 2.69±0.12, 60^{th} day- 4.94±0.01, 90^{th} day- 6.95±0.04 µmol/mg of protein) *A. platensis* incorporated feed fed group exhibited a higher activity when compared to control group. Histological studies: Various concentrated *A. platensis* meal treated shrimp stomach, HP, Gut and Gills showed no significant morphological changes. *A. platensis* 50% incorporated feed fed groups HP showed increase number of B cells (Producer of Digestive enzyme) and shrimp midgut microvilli size and thickness also increased (Increase the absorption activity) with compared to control group.

Maximum survival rate was observed in 50% replaced feed when compared with other feeds (Control- 98%, Pathogen control- 40%, 25% feed - 52%, 50% feed - 78%, 75% feed - 50%, 100% feed - 43%). Results revealed that pathogen challenged group ($3.8x10^8$/ml) showed increasing number of Total hemocyte count. Different percentage replaced *A. platensis* meal treated groups (25%- $4.3x10^7$/mL, 50%- $4.8x10^7$/mL, 75%- $3.1x10^7$/mL and 100%- $3.3x10^7$/mL) were result minimum significant changes on Total Hemocytes count when compared to the control ($1.8x10^7$/ml) groups. Histopathological studies on pathogen challenged shrimp HP, Gut and Gills showed severe significant pathological changes were observed. *A. platensis* treated groups results minimum pathological changes. Pathogen challenged HP showed severe necrosis, reduce the R cell activity with Increase the haemal sinus space with hemocytic congestion. Treated group HP showed increased R cell activity with mild hemocytic infiltration. Treated group (50%) Shrimp mid-gut microvilli size and thickness also increased (Increase the absorption activity) with compared to pathogen challenged group.

4. Conclusion

In this study, the non-enzymatic antioxidants were significantly elevated in experimental feed fed group. The elevation was recorded higher in 50% incorporated feed. Enzymatic antioxidant SOD, CAT and LPO showed no variation in experimental group when compared with control. Hence, the present results revealed that the feeds were well utilized by the shrimp and do not produce any toxic effects. The present study concluded that the replacement of fishmeal with 50% of *Arthrospira platensis* meal considered as a potential candidate species for further application used as an aqua feed additive to replace the high cost fish meal. The outcome of the challenging test including Hematology, Histopathology and Antioxidant activities of shrimp fed with 50% *Arthrospira platensis* incorporated feed shows the better survival rate compared to other groups.

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Chapter: 6

Climate-Smart Fish Feed: Designing sustainable diets for fish reared at high temperature

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1. Introduction

Global warming pose serious threat to the poikilothermic organisms such as fish by altering their body temperature and thereby disrupting physiological and metabolical homeostasis. Among abiotic factors which determine fish production, water temperature is the most relevant environmental factor, playing a crucial role in metabolism and nutrient utilization in fish. Higher water temperatures can increase the metabolic rates of fish. This can lead to increased energy requirements for basic physiological functions such as growth, reproduction, and maintaining homeostasis. Most fishes can acclimate to gradual changes in temperature or adapt over generations through evolutionary processes. Prolonged or extreme thermal stress can overwhelm the protective mechanisms in fish, leading to cellular damage, metabolic disruptions and thereby compromising the health and survival of fish species. Changes in water temperature can also affect the timing of reproductive cycles in fish species, potentially disrupting breeding seasons and impacting population dynamics, which in turn reduces fertility rates and egg quality. However, once the organism survives the above optimal temperature, the next challenge is to maintain the growth of the animal. From aquaculturists' point of view, the most important input affecting the growth is feed and its utilisation at high temperature has to be given critical consideration while formulating feed. Thus, a sustainable aquafeed is pivotal in the context of global warming and fish farming because it directly influences the environmental sustainability, economic viability, and resilience of aquaculture operations. By optimizing nutrition, supporting immune function, reducing stress, and promoting environmental adaptation, thermoresilient diets play a critical role in ensuring the long-term sustainability and success of aquaculture operations worldwide.

2. Role Of Essential Nutrients In Thermal Tolerance

The utilisation of major energy nutrients, proteins, lipids and carbohydrates are found to be altered with the changes in temperature. For instance, a higher hepatic lipid deposition at low temperature has been observed for many fishes. Similarly, a higher utilisation of carbohydrates have alos been observed during the rearing at stressful conditions in fish. Reentl, it has been observed that a high lipid diet perform better than a high protein diet at higher temperatures. While each nutrient plays a crucial role in alleviating thermal stress in the context of climate change, lipids (fats and oils) are typically favored as the primary energy source by fish. Lipids provide a concentrated source of energy, containing more than twice the energy content per unit weight compared to proteins and carbohydrates. Additionally, fish can efficiently metabolize lipids and store them as energy reserves, which is advantageous for maintaining metabolic functions and supporting growth, especially during periods of reduced food availability or environmental stress, like elevated water temperatures. Lipids are advantageous also due to their low specific dynamic action (SDA) and higher digestibilities, which minimizes metabolic costs and enhances their efficiency as a readily utilizable energy source. However, the specific preference for energy sources can vary among fish species based on their natural diet, habitat, and physiological adaptations.

Proteins are also essential for growth, tissue repair, enzyme function, and immune response regulation. During thermal stress, proteins are vital for maintaining cellular structure and function. They play a key role in stabilizing cell membranes and regulating osmotic balance, which are critical for coping with temperature fluctuations. They also support the synthesis of heat shock proteins (HSPs), which help protect cells from damage caused by heat stress. Lipids serve as energy reserves, structural components of cell membranes, and carriers of fat-soluble vitamins and essential fatty acids. Essential fatty acids (EFAs), such as omega-3 and omega-6 fatty acids, derived from lipids are crucial during thermal stress. They help maintain membrane fluidity and integrity, which is essential for cellular function under varying temperatures.

Carbohydrates serve as a primary energy source and provide structural support in cell walls (e.g., in algae and some shellfish). During thermal stress, carbohydrates contribute to energy metabolism, supporting increased energy demands for adaptation and recovery. They also play a role in osmoregulation and maintaining cellular integrity under these changing climate events.

Micronutrients, specifically vitamins and minerals, play essential roles in thermal stress response and immune function in aquatic organisms within aquaculture settings. Vitamins are essential for energy metabolism, nerve function, and stress response. They play crucial roles in maintaining metabolic pathways under thermal stress. Minerals also have antioxidant immunomodulatory functions.

3. Pecific Additives for Enhancing Thermo-Resilience

Aquafeed additives aimed at enhancing thermal tolerance play a crucial role in improving the performance of aquatic organisms under hyperthermal habitats.

3.1 Antioxidants

Antioxidants shield cells from oxidative stress triggered by heat, which helps maintain cellular integrity and function. This protection can be provided by natural antioxidants such as vitamin C, vitamin E, and carotenoids, as well as synthetic antioxidants.

3.2 Vitamin C and its role in stress mitigation

Vitamin C, also known as ascorbic acid, plays a significant role in combating thermal stress in fish primarily through its antioxidant and physiological functions. It is a potent antioxidant that scavenges free radicals and reactive oxygen species (ROS) generated during thermal stress. Vitamin C is also essential for collagen synthesis, a structural protein that is crucial for maintaining the integrity of connective tissues, including skin, bones, and blood vessels. During thermal stress, collagen turnover may increase to repair damaged tissues. Adequate levels of vitamin C support this process, aiding in tissue repair and resilience.

3.3 L-Tryptophan as a stress alleviator

Fish have different requirement levels for L-tryptophan depending on their species, stage of life, and habitat. However, the requirement of tryptophan remains lower than the other essential amino acids. Tryptophan is an essential amino acid that is a precursor of serotonin, a neurotransmitter involved in fish physiological control and stress responses. Serotonin reduces cortisol levels, where the highlevel of the latter can suppress the immunity of the organism in the long term.

Dietary Tryptophan can promote growth, moderate aggressive behaviour, or lessen cannibalism as well as stress-induced anorexia and cortisol surge in a variety of fish species. Because tryptophan and its metabolites are antioxidants, thus L-tryptophan supplementation in aquafeed can potentially enhance the fish's ability to withstand thermal stress and maintain cellular integrity (Zhang et al., 2017). Studies in Nile tilapia indicate that tryptophan supplementation may improve immunological response and lower indicators of oxidative stress after episodes of heat stress. L-tryptophan also improves immunological responses to infections and environmental stressors by influencing immune cell activity and cytokine production (Jobling, 2012). Thus, L-tryptophan incorporation into aquafeed can significantly enhance fish welfare, performance, and sustainability in aquaculture practices.

3.4 Osmolytes and Osmo protectants

Osmolytes and Osmo protectants are essential compounds that help aquatic organisms combat thermal stress by regulating osmotic balance, stabilizing cellular structures, and minimizing physiological damage. Taurine is an amino acid derivative that functions as an osmolyte in various aquatic organisms. It helps regulate cell volume, stabilize membranes, and protect against oxidative stress induced by thermal fluctuations. Proline is an amino acid that accumulates in cells during osmotic stress and thermal stress. It stabilizes protein structures, maintains cell integrity, and contributes to antioxidant defences.

Sorbitol is a sugar alcohol that serves as an osmoprotectant by regulating the osmotic pressure and protecting cells from dehydration during thermal stress. Mannitol is another sugar alcohol used by some aquatic organisms as an osmoprotectant. It helps maintain cellular turgor and stabilize membranes under heat-stress conditions.

Betaine, also known as trimethylglycine, is a naturally occurring compound found in many organisms. It's a zwitterionic quaternary ammonium compound with a fully methylated nitrogen atom. Betaine acts as an organic osmolyte, helping to maintain cell volume and fluid balance. It accumulates in cells without disrupting cellular functions, unlike inorganic ions.

By acting as an osmolyte, betaine reduces the energy required for ion pumping, which is crucial during stress periods. Betaine can act as a methyl donor in various metabolic processes, potentially improving overall metabolic efficiency under stress. Typical inclusion rates range from 0.1% to 2% of the diet, but optimal levels can vary by species and condition. Some feed ingredients, like wheat and sugar beet, naturally contain betaine. However, supplementation is often necessary to achieve protective levels.

3.5 Immunostimulants

Immunostimulants play a pivotal role in boosting fish immune responses, especially amidst thermal stress conditions where both high and low temperatures can compromise immune function, rendering fish more susceptible to pathogens. These substances, such as microbial derivatives (e.g., β-glucans, lipopolysaccharides), plant extracts (like aloe vera, ginger, and garlic), nutritional factors (including vitamins C and E, zinc, and selenium), synthetic chemicals (such as levamisole), and probiotics/prebiotics, bolster immune system functionality. They achieve this by enhancing phagocytic activity, increasing lysozyme and complement activity, stimulating antimicrobial peptide production, promoting lymphocyte proliferation, and boosting cytokine and immune mediator production.

3.6 Probiotics and prebiotics

Probiotics and prebiotics play a crucial role in supporting gut health and nutrient absorption in fish, particularly under warm conditions. Fish gut health is crucial for nutrient absorption, immune function, and overall fish health. Warm waters can affect gut microbiota composition and function. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Common probiotic strains for fish include *Lactobacillus* sp., *Bacillus* sp., *Enterococcus* sp., *Saccharomyces cerevisiae* etc.

Prebiotics are non-digestible food ingredients, particularly carbohydrates, that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial gut bacteria. Common prebiotics for fish include Inulin, Fructooligosaccharides (FOS), Mannan oligosaccharides (MOS), and Galactooligosaccharides (GOS). They promote the growth of beneficial bacteria by serving as a substrate, enhance nutrient digestibility by modulating gut pH, enhance short-chain fatty acid production, improve gut morphology, enhance mineral absorption, and support immune function in thermally stressed fish.

Typical inclusion rates vary but generally range from 106 to 108 colony-forming units (CFU) per gram for probiotics and 0.1% to 2% for prebiotics in fish feeds. By fostering a balanced gut microbiome, improving digestive efficiency, and supporting overall health, these substances contribute to enhancing fish resilience against the challenges associated with higher temperatures.

3.7 Enzymes

Enzymes play a crucial role in improving digestibility and nutrient utilization in fish, especially under varying temperature conditions. Fish produce various endogenous enzymes for digestion, including proteases, lipases, and carbohydrates. Supplemental enzymes can support the increased digestive demands. Improved protein digestibility was observed in Atlantic salmon-fed protease-supplemented diets across seasonal temperature changes. As aquaculture continues to face challenges from climate variability, strategic use of enzymes in fish feeds may become an increasingly important tool for sustainable and efficient fish production.

3.8 Essential fatty acids

Essential fatty acids (EFAs) play a crucial role in fish nutrition, and their importance becomes even more significant in the context of thermal stress and climate change scenarios. The main EFAs for fish are: a) Omega-3 fatty acids: EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) b) Omega-6 fatty acids: primarily arachidonic acid (ARA). Temperature changes may alter the optimal dietary EFA ratios for different fish species. Marine fish generally have a lower capacity to synthesize long-chain PUFAs from shorter-chain precursors, making dietary sources more critical.

3.9 Omega-3 fatty acids for membrane stability

Omega-3 fatty acids play a crucial role in maintaining membrane stability in fish as rearing water temperatures rise. Cell membranes are composed of a phospholipid bilayer. The fatty acid composition of these phospholipids greatly influences membrane properties. The highly unsaturated EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) contribute to the membrane fluidity. As temperature increases, membranes become more fluid. Excessive fluidity can lead to membrane instability and dysfunction. Fish can adjust their membrane composition to maintain proper fluidity across temperature ranges. This process often involves changing the ratio of saturated to unsaturated fatty acids. Omega-3s can help prevent excessive membrane permeability during heat stress.

3.10 Minerals and trace elements

Incorporating bioavailable mineral sources in aquafeed formulations ensures optimal absorption and utilization by fish, maximizing the benefits of mineral supplementation in mitigating thermal stress. Fish energy generation and utilisation are influenced by several metabolic pathways that include calcium (Ca) and magnesium (Mg). Temperatures above normal might change metabolic rates and raise energy needs. Sufficient mineral concentrations facilitate metabolic adaption to elevated temperatures, guaranteeing effective energy utilisation for essential activities like swimming, eating, and preserving physiological equilibrium (Iversen and Findorf, 2013).

Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) depend on minerals like selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn). According to Ozório et al. (2020), these enzymes shield cells from oxidative damage brought on by reactive oxygen species (ROS) produced by heat stress. Zinc induces the synthesis of metallothioneins, which are small, cysteine-rich proteins. Metallothioneins can directly scavenge free radicals and also serve as zinc reservoirs. Selenium is an essential component of Glutathione Peroxidase (GPx), incorporated into the enzyme as selenocysteine. GPx detoxifies hydrogen peroxide and lipid hydroperoxides, protecting cellular membranes from oxidative damage.

Minerals like magnesium (Mg) and zinc (Zn) have a role in controlling the synthesis and sensitivity of stress hormones like cortisol. According to Mommsen et al. (1999), cortisol has a crucial role as a mediator of the stress response in fish, affecting their physiological and behavioural responses to various environmental stressors, such as temperature variations.

4. Sustainable Sourcing of Aquafeed Additives

Criteria for sustainable aquafeed ingredients in the context of climate change

In the context of climate change, sustainable aquafeed ingredients need to consider a balance of environmental, nutritional, economic, and social factors. They must have minimal environmental impact, be adaptable to changing climatic conditions, provide essential nutrients, and be cost-effective. It's crucial to decrease reliance on marine-based ingredients like fishmeal and fish oil by integrating sustainable alternatives such as protein and lipid sources from non-marine origins that offer nutrition and sustainability benefits. It's essential to source ingredients from responsibly managed fisheries and aquaculture operations to avoid contributing to overfishing or habitat destruction.

4.1 Algal and insect-based proteins

Algal and insect-based proteins are emerging as promising alternatives to traditional fishmeal in aquaculture feeds, particularly in the context of climate change. Algal and insect-based proteins ensure lower carbon footprints compared to traditional crops, potential for carbon sequestration during growth and can be produced in controlled environments. They also encompass the comparative advantage of higher protein yield per unit area than the traditional ingredients with a potential for vertical farming and urban production. However, challenges in scaling production, optimizing nutritional profiles, and gaining regulatory approval need to be addressed.

4.2 Sustainable oils and fats

Fatty acids play pivotal roles in the physiology of warm-water species. They serve as essential energy sources and contribute significantly to the structure and function of cell membranes. They are vital for supporting growth, reproductive health, and immune system functions in aquatic organisms. Additionally, the composition of fatty acids directly impacts membrane fluidity, a critical factor in thermal adaptation. This flexibility is indispensable for warm-water species as they adjust to and thrive in environments with higher water temperatures, ensuring their physiological resilience and overall survival in changing aquatic conditions. The use of glycerol as a lipotropic additive is also found to be economically beneficial for fish farming.

Warm-water species, such as tilapia, carp, and catfish, typically require lower levels of long-chain omega-3 fatty acids like EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) compared to cold-water species. Strategies like oil blending, tailored formulations, and finishing diets can help achieve the desired fatty acid profiles while minimizing reliance on unsustainable sources.

5. Case Studies and Experimental Approaches

Research findings demonstrate that thermoresilient diets can significantly improve fish performance, health, and survival under thermal stress conditions. However, the optimal formulation varies depending on species, life stage, and specific environmental conditions.

5.1 Thermoresilient diet development for salmonids

Studies have explored the role of amino acids such as arginine and methionine in enhancing the thermoresilience of Atlantic salmon. These amino acids contribute to antioxidant defences and protein synthesis pathways, supporting physiological adaptation to thermal stressors in aquaculture settings (Kullgren et al., 2013). Atlantic salmon-fed diets enriched with antioxidants (vitamin E, vitamin C, and selenium) improved survival rates and reduced oxidative stress markers during heat stress (Hamre et al., 2014). Rainbow trout-fed diets containing higher levels of omega-3 fatty acids, particularly EPA and DHA, exhibit improved thermal resilience. These fatty acids enhance membrane fluidity and stability, crucial for maintaining cellular function and adapting to fluctuating water temperatures (Roy et al., 2020).

5.2 Aquafeed additives for warm-water species

Research has demonstrated that diets enriched with antioxidants such as vitamin E and selenium can enhance the thermotolerance of tilapia. These antioxidants protect cellular membranes from oxidative damage caused by high temperatures, thereby improving overall health and survival rates in warm water conditions (Rohani et al., 2023). Increased dietary protein: energy ratios improved growth and feed efficiency in Nile tilapia under high-temperature conditions (Qiang et al., 2013).

The inclusion of herbal extracts (e.g., ginger, garlic) in diets of the same species enhanced immune function and stress resistance at elevated temperatures (Mahmoud et al., 2019). Diets supplemented with specific nutrients like vitamin C and probiotics can improve the thermotolerance of channel catfish. These additives help mitigate oxidative stress and support immune function, enhancing survival and growth during heat stress events (Liang et al., 2015; Hafsa et al., 2023).

5.3 Additives for Sea bass and seabreams

Taurine supplementation improved growth performance and reduced oxidative stress in European sea bass exposed to high temperatures (Feidantsis et al., 2014). Diets with optimized amino acid profiles enhanced protein retention and growth under thermal stress in the species (Richard et al., 2016). In gilthead sea bream, dietary inclusion of nucleotides improved immune response and stress tolerance during temperature fluctuations (Hossain et al., 2016). Also, supplementation with organic selenium enhanced antioxidant capacity and reduced lipid peroxidation under heat stress (Tseng et al., 2024).

5.4 Shrimp feed formulations for high-temperature environments

Simon (2024) suggests that optimizing protein levels and balancing amino acid profiles in shrimp feeds can improve growth and survival and support metabolic processes and immune function while reducing nitrogen waste under high-temperature conditions. Incorporating specific lipid sources rich in omega-3 fatty acids, such as fish oil and algae oil, helps maintain membrane integrity and enhances tolerance to heat stress in shrimp (Coelho et al., 2023).

Effective use of digestible carbohydrates, such as wheat and corn, in shrimp diets supports energy metabolism and reduces the risk of digestive disorders in warm-water species (Molina-Poveda et al., 2015). Antioxidant additives like astaxanthin, vitamin C, and vitamin E are commonly included in shrimp feeds to combat oxidative stress caused by high temperatures (Chien et al., 2003; Le et al., 2021).

6. Future Directions and Challenges

Challenges in developing thermoresilient diets with suitable additives include the complexity of nutritional requirements across diverse fish species, the economic viability of novel feed ingredients, and the regulatory framework. Conducting comprehensive research to understand how different fish species respond to rising water temperatures and other climate stressors is the need of the hour. Overcoming the above challenge requires embracing technological innovations such as precision feeding systems, microencapsulation of nutrients, and biotechnological approaches to enhance feed efficiency and nutrient utilization. These technologies can optimize the delivery of essential nutrients while minimizing environmental impact.

7. Conclusion

At present several osmo protectants and additives like vitamin C, vitamin E, astaxanthin, Betaine, Taurine, essential amino acids, exogenous enzymes, fatty acids, herbal extracts etc. have been tested to improve thermal resilience in fishes. Together, these additives contribute to sustainable aquafeed formulations that promote health, growth, and resilience in aquatic species facing climate-related challenges. However, the scope for exploration is unlimited. Collaboration among researchers, industry stakeholders, and policymakers is essential to drive innovation, ensure regulatory compliance, and promote global adoption of sustainable aquafeed practices aimed at mitigating the impacts of climate change on aquaculture.

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Chapter: 7

Fish Nutritional Requirements and Eco-Friendly Feed Formulation through Bio-Conversion Process

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1. Introduction

Culture of aquatic organisms from fry or fingerlings up to marketable size in a controlled environment is called aquaculture. The allied aspects such as seed production through hatchery, feed formulation and production, harvesting and marketing will also come under aquaculture practice. The main objectives of aquaculture are (i) to substantiate the stagnated natural fishery production as well as to ensure protein rich food at a reasonable price to the countries undernourished population, (ii) to enhance export earnings and (iii) to create employment opportunities to the skilled and unskilled rural people. There are different types of aquaculture system, which includes: traditional aquaculture farming, extensive farming, semiintensive farming, intensive farming and super intensive farming. The advancement of these culture systems necessitate artificial feeding, because no availability of natural feeds in intensive and super intensive farming's. More over good nutrition in fish culture is essential to the economic production of a healthy, high-quality product with rich survival, growth, reproductive performance, spawning success, and body composition. In aquaculture, nutrition is critical because feed typically represents approximately 50 % of the variable production cost. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health (Makode, 2017). Jhingran (1991) noted that formulated feed plays a vital role in semi intensive fish culture, where it is required to maintain a high density of fish than the natural fertility of water. Thus supplementary feed can be considered as a most desirable measure for increasing fish yield.

Nowadays, feed costs make up a significant fraction of total fish production costs. Optimizing growth rates and feed efficiency in fish depends on the way in which feed is made, availability of feed, the amount of food delivered, feeding frequency, timing of each feeding periods and the characteristics of the diets (Ali, and Hoq, 2010). In most traditional aquaculture practices, herbivorous or omnivorous species have been preferred as they feed on natural food in water. Thus growth can be easily enhanced through cheap supplementary feeding and fertilization. But carnivorous species such as catfishes, murrels, etc. need a high protein diet. New and Wijkstrom (2002) mentioned that on the basis of growth rate, feed conversion, and nutrient retention is protein, lipid, carbohydrate and gross energy. Among all the nutrients, Protein is by far the essential nutrient needed for growth being efficiently utilized by several fish species and is very expensive. An inclusion levels, quality of protein and the ratio of protein to another dietary nutrient significantly alter the growth pattern of fish (Rodge *et al*., 2020). Traditionally, fish meal is the preferred dietary protein source for many farmed fish species due to its amino acid profile, vitamin mineral content, high digestibility, palatability and unidentified growth factors and is currently the primary source of protein fed to farm fishes.

2. Feed in aquaculture systems

The aquaculture feed can be classified as natural feed (Phyto & Zoo plankton) and artificial feed (Supplementation of formulated feed). The artificial feed is given when the aquaculture system is to be advanced like semi intensive, intensive or super intensive. Depending on the advancement of the aquaculture system, the stocking density (SD) of seeds will be increased, for eg. semi intensive-5/m^2, intensive – 20/m^2 and super intensive- >20/m^2. Similarly depends on the advancement of aquaculture system, the availability of natural feed is decreased in semi intensive farming and it is almost nil in intensive or super intensive farming. Likewise supplementation of artificial feed is depending on the aquaculture practice and simultaneously, it is increased from semi intensive to super intensive farming's. Thus stocking density, availability of natural feed and supplementation of artificial feed are inter related and these three will be increased based on the system development and ultimately there will be the chances of enhancement of capital and running cost, susceptibility to disease and its level of management (Joshi *et al*., 2021).

2.1. Types of artificial feed

Depending upon the types of feed ingredients used for feed formulation, it can be classified the artificial feed in to two types (i) Simple feed (non-balanced or single supplement feed, eg. Fish meal/Soy powder, ground nut oil cake, etc. alone) and (ii)

Compound feed (balanced feed or complete feed, eg. plant & animal based ingredients). Based on the feed processing artificial feeds are classified into the following types (Fig.1).

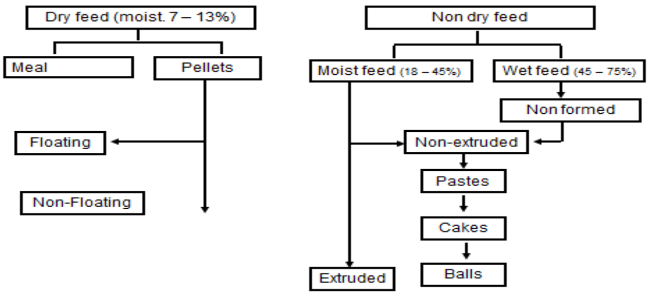


Fig.1. Types of artificial feeds

3. Nutritional requirement in fishes

Normally the fry and fingerlings of fishes will feed on plankton (phyto and zooplankton), which are abundant in well fertilized ponds. However, the natural foods in ponds become a limiting factor in the intensive culture systems. Hence, supplementary feeding has become a common practice. Fishes are the efficient converter of food than terrestrial organisms. According to feeding behaviour, the fishes are classified as (i) Herbivore fishes (*Chanos chanos*), (ii) Carnivore fishes (*Salmon, Anguilla* sp. etc) and (iii) Omnivore fishes (*Oreochromis mossambicus*). Fishes will feed on food materials to satisfy their protein requirement. Hence, protein is the important constituent as for as fish feed is concern. For the better digestion and assimilation of dietary protein, appropriate concentration of carbohydrate and lipid are also needed. Therefore, the composition of macronutrients in the diet will determine the efficiency of diet. Available information on the qualitative and quantitative aspects of fish nutrition has been reviewed by Pandian (1987). It has been established that protein is required by all animals for body maintenance and growth, and that the protein level for these functions varies with species and culture environment.

Protein requirement of fish also varies with body weight, for eg. Channel catfish of 4 - 100g require 35% protein diet or 114 - 500g requires 25% protein diet; whereas the dietary energy is same in both the cases. In rainbow trout (*Salmo gairdneri*), low protein-energy diet resulted poor growth, but high protein-energy density beyond the optimum level also did not support better or faster growth. Therefore for fish, the optimal amount of protein in feed is important, because either a low or high protein level may result in poor growth and increased susceptibility to disease. Additional excessive protein content in fish diet is wasteful and cause the diets to be unnecessarily expensive. The quantitative requirement of any food item depends largely on its composition; the most efficient level of feeding is attained only in correct supply of energy and essential nutrients in feed for better growth. Any deviation from the ideal composition will also change the quantitative food requirement. Protein requirement of certain herbivorus, carnivorus, and omnivorus fishes are given below (Pandian, 1987 modified) (Tables 1-3)

Table 1. Protein requirements of some carnivore fishes

Species	Tested diet	Mid body wt (g)	Feeding rate (% body wt day^{-1})	Optimum protein (% dry wt of food)
Anguilla japonica	Casein	4.4	*ad lib*	45
A. anguilla	-	-	-	62
Channa micropeltes	Fish meal	21.0	1.8	52
Chrysophrys aurata	Casein / amino acids	8.9	1.8	52
C. major	-	-	-	55
Fugu rubripes	Casein	3.2	3.2	47
Norone saxatilis	Fish / soy meal	6.0	2.8	55
Oncorhynchus tahawytsacha	-	-	-	48
Pleuronectes platessa	Cod muscle	22.3	1.8	50
Salmo gairdneri	Casein / gelatine	12.6	Ad lib / 1.7	43
S. salar	-	-	-	40
Salvelinus alinus	Fish meal	-	-	40
Seriola quinqueradiata	-	-	-	50
S. gairdneri	Tubifex / pellet	3.5	2.5, 4, 5.5	-
S. gairdneri	Pellet	6.5	5.0	40.7
Oncorhynchus kisutch	Pellet	4.3	-	51

Table 2. Protein requirements of some herbivore fishes

Species	Tested diet	Mid body wt (g)	Feeding rate (% body wt day^{-1})	Optimum protein (% dry wt of food)
Chanos chanos	Casein	0.11	8.2	39
Ctenopharyngodon idella	Casein	0.34	-	42
Tilapia mossambicus	Fish meal	5.1	4.7	42
T. mossambicus	Fish meal	6.3	3.0	35
T. niloticus	Fish meal	0.31	6.4	35
Oreochromis niloticus	Pellet	0.185 - 1.08	2.5 – 5.5	30
Sarotherodon mossambicus	Pellet	5.1	6.0	40

Table 3. Protein requirements of some omnivore fishes

Species	Tested diet	Mid body wt (g)	Feeding rate (% body wt day^{-1})	Optimum protein (% dry wt of food)
Cyprinus carpio	Fish meal	13.6	2.9	54
C. carpio	Pellet	150	-	-
Ictalurus punctatus	-	-	-	40
I. punctatus	Pellet	24	2.6	40
I. punctatus	Hermetia illucens / pellet	-	-	30
Micropterus salomoides	Fish / gelatine / amino acids	3.5	2.9	40
M. dolomieui	Fish / gelatine / amino acids	3.6	2.4	45
M. salmoides	Pellet	14.4	-	35.8
M. salmoides	Pellet	14.4	-	35.9
Tilapia aureus	Fish / soy meal	4.4	3.5	36
T. aurea	Caesin / albumin	2.5	2.0	36
T. aurea	Casein / albumim	2.5	10.0	34

4. Feed Formulation

The formulation of a diet represents the translation of energy and nutrient requirements into a balanced mixture of feed ingredients for a group of animals. This formulated diet should then meet the daily need of energy and nutrients to support the maintenance and growth of the animals. There are four major parameters to be considered during feed formulation such as ingredients, binders, antioxidants and preservatives (Sarker *et al*., 2020)

a. Ingredients: It is essential to know (i). What are the feed ingredients to be used? (ii). Availability of the raw materials, (iii). Important plant raw materials and (iv). Their proximate composition

b. Binders (0.5 to 5.0%): (i). What are binders? (Materials having gelatinisation effect to bind the ingredients), (ii). Kinds of binders like natural binders (Seaweed, tapioca powder, wheat flour), Artificial binders (gum arabic 1-2%) and Synthetic binders (Guar gum, Alginates, gelatin & CMC)

c. Antioxidants (0.1 – 0.5%): eg. BHA, BHT and Vitamin C

d. Preservatives (0.1%): eg. Sodium benzoic acid, Potassium benzoic acid, Calcium benzoic acid, Sodium sorbic acid, Potassium sorbic acid and Calcium sorbic acid

4.1. Selection of the ingredients

Both plant and animal based ingredients should be available locally throughout the year. The cost of the feed ingredients must be very low to formulate cost effective feed. It should solve the basic needs in feed formulation such as protein, carbohydrate and lipid supplements, it may act as a binder or it may also act as a attractant, it should have more bio-available nutrition (high digestibility, efficient for easy digestion and assimilation by candidate species). It should be free from metal and pesticide contamination and it should be free from pathogenic microbes (harmful bacteria and fungi) (Shukla *et al*., 2019)**.** Availability of marine animal raw materials and their proximate composition, availability of plant based raw materials from India as well as the nonconventional feed materials are given below (Tables 4-7)

Table 4. Availability of marine animal raw materials in India (Source: Ali *et al.,* 1998)

Sl. No.	Raw materials	Estimated quantity (t/yr) (dry weight)
1	Fish meal	4,000
2	Dry fish	1,76,600
3	Mantis shrimp	57,900
4	Prawn head waste	31,200
5	Small crabs	6,100
6	Sergested shrimp (Acetes)	48,000
7	Squid	7,000
8	Cuttle fish	3,900
9	Clam meat	3,700
10	Mussel meat	800
11	Fish oil	1,000
	Total	**3,40,200**

Table 5. Typical proximate composition of raw material (Source: Ali *et al.,* 1998)

Raw material	Crude protein (%)	Fat (%)	Carbohydrate (%)
Fish meal	55.4 – 59.9	1.7 – 3.8	0.8 – 1.0
Pulverised fish powder	45.4 – 50.7	5.6 – 6.8	6.1 – 8.1
Imported fish meal	68.0 – 74.0	1.0 – 8.1	0.9 – 1.9
Squid meal	68.1	4.9	1.9
Squilla meal	43.1	6.9	1.9
Prawn head meal	34.8	9.6	4.1
Clam meat meal	48.4	13.7	14.3
Mussel meal	48.4	18.7	19.6

Table 6. Important plant raw materials available in India (Source: Ali *et al.*, 1998)

Name of the material	Estimated quantity (in metric tonnes)
Protein sources	
Soy bean cake (CP 45 – 50)	8,38,600
Groundnut cake (CP 40 – 45%)	43,47,300
Sun flower cake (CP 22 – 28%)	1,78,200
Sesame cake (CP 30 – 35%)	26,200
Coconut cake	47,200
Rape seed cake	7,67,700
Cotton seed cake	7,50,000
Rice bran	2,83,000
Wheat bran	12,00,000
Carbohydrate sources	
Wheat	2,00,00,000
Tapioca	56,00,000
Millets	1,90,00,000

Table 7. Non-conventional feed materials (Source: Ali *et al.,* 1998)

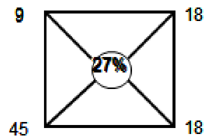
Name of the product	Crude protein (%)	Inclusion rate (%)
Meat and bone meal	45 – 50	8 – 15
Blood meal	80 – 85	5 – 10
Poultry waste	-	15 – 20
Poultry feather meal	80 – 85	5 – 10
Silkworm pupa meal	45 – 60	10 – 15
Single cell protein (Spirulina)	60	2 – 8
Yeast	40 – 45	2 – 5
Cyanobacteria	45 - 60	-

4.2. Methods in feed formulation

There are two methods mainly employed for feed formulation (i) Square method and (ii) Linear programming method. In square method, based on the fixed protein level, again two methods can be followed, they are Square method for two ingredients and Square method for more than two ingredients.

4.2.1. Square method for two ingredients: Consider rice bran (9% crude protein) and groundnut oil cake (45% crude protein) are two ingredients for making 27% protein diet for fish and it can be prepared by following steps:

- A square is constructed and the two ingredients are placed on the two left corners along with the protein content of the same.
- The desired protein level of the feed is placed in the middle of the square.
- Next the protein level of the feed to be formulated (27%) is subtracted from that of the ingredients and the answer is placed in the opposite corner (In this stage, the positive (+) or negative (-) sign can be ignored).



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- Then add the figures in the right side corner of the square: 18 + 18 = 36
- Now you can calculate the percentage of rice bran: 18 / 36 x 100 = 50%
- By the same way, calculate the percentage of groundnut oil cake: 18 / 36 x 100 = 50%
- To make 100g of feed, take 50 g of rice bran and 50g of groundnut oil cake.
- If you measure the crude protein of the prepared feed by using both ingredients, it should be 27%

4.2.2. Balancing protein more than two ingredients:

Ingredients used:

- Prawn waste (PW) C. P. = 35%
- Fish meal (FM) C. P. = 60%
- Wheat bran (WB) C. P. = 13%
- Tapioca (TP) C. P. = 2%

A prawn diet with 32% crude protein (C.P) can be prepared as follows.

(i) Grouping of ingredients:

- Group the ingredients less than 20% C.P. and average their protein content.

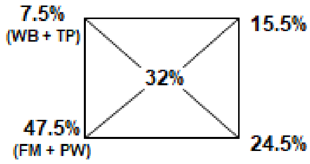
  WB = 13% and TP = 2%; Total (15/2) = 7.5% (average)

- Group the ingredients more than 20% C. P. and average their protein content.

FM = 60%, PW = 35%; Total (95/2) = 47.5% (average)

(iii) Now can follow the square method

The average protein contents are put in the two left corners of the square and the rest of the calculations are same as that followed for two ingredients



- Now add the figures on the right hand side of the square: 15.5 + 24.5 = 40.0
- For first combination (C. P. < 20%): WB + TP = 15.5 / 40 x 100 = 38.75%
- For second combination (C.P. > 20%): FM + PW = 24.5 / 40 x 100 = 61.25%

- Now calculate the amount of each ingredients

  » WB = 38.75 / 2 = 19.375%

  » TP = 38.72 / 2 = 19.375%

  » FM = 61.25 / 2 = 30.625%

  » PW = 61.25 / 2 = 30.625%

- The composition of feed contains 100g

  » PW = 30.625

  » FM = 30.625

  » WB = 19.375

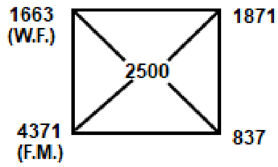
  » TP = 19.375

  Total = 100.000

4.2.3. Balancing energy

The square method can also be used to calculate the proportion of feed ingredients to be mixed together to achieve a desired dietary energy level.

By using wheat flour of 1663 k.cal/kg and fish meal of 4371 k.cal/kg, a feed containing 2500 k.cal/kg can be prepared using square method



- Wheat flour = 1871 / 2708 x 100 = 69.1%
- Fish meal = 837 / 2708 x 100 = 30.9%

To make 100 kg of feed with 2500 k.cal/kg, we have to mix 69.1 kg of wheat flour and 30.9 kg of fish meal. The square method cannot be used to balance for both CP level and energy level simultaneously. For simultaneously balancing protein / energy in the diet, a method called Linear Programming has to be followed.

4.3. Aquaculture feed production

4.3.1. Procedure for feed manufacture

Weigh all the ingredients and mix thoroughly, to this add 130 -135 ml of distilled water and mix again. Adjust the pH of the diet to pH 6.8-7.0 with 0.5N NaOH (if necessary), then heat the mixture in autoclave (without giving pressure) at 100^0C for 10 min. After heating take the container from the autoclave and mix the ingredients, then bring this to room temperature and add vitamins and minerals (or) other supplements if necessary and mix these thoroughly (Supplementation). Make this as dough in the form of noodles using a pelletizer or extruder. Finally dry the pellet feed under sun light or hot air oven.

4.3.2. Feed analysis

The quality of the prepared feed must be important in the aquaculture point of view by knowing its proximate composition as well as Feed conversion ratio (FCR), Specific growth rate and other bio-energetic parameters. The proximate composition of the feed can be determined by analysing its moisture content, protein content, carbohydrate content (Fibre and Nitrogen free extract), lipid content and Ash content by following appropriate methodologies.

After feeding experiment for the specific time period, the Feed Conversion Ratio (FCR) can be estimated by the following formula

$$FCR = \frac{\text{Amount of feed given to the fish (dry wt)}}{\text{Weight of the fish harvested (Wet wt)}}$$

FCR may be varied as 1: 1.5 (or) 1: 1.2 (or) 1: 2

For eg. expression ratio of 1.2: 1 is 1.2 unit of feed is required to produce 1 unit of fish, thus, lesser the FCR value higher the efficiency (FCR in the order of 2: 1 < 1.5: 1 < 1.2: 1).

4.3.3. Problems in artificial feed

The following are the major problems usually encountered in the prepared aquaculture feeds

(i). Poor water stability (Due to high grain size, improper binding & cooking)

(ii). Loss of nutrients (Leeching effect)

(iii). Microbial contamination (Due to excess moisture-pathogenic microbes grow and produce toxins)

(iv). Low FCR value (Due to the above i-iii factors & feed waste)

(v). Feed related environmental problems (Pollution due to ammonia, Co_2, H_2S& Phosphate)

(vi). Antinutritional factors (Chemical compounds present in the feed ingredients will inhibit the feeding behaviour of fish, inhibiting enzyme action involving digestion, eg. b-glucan, phytic acid, tanic acid etc.)

(vii) Non-palatability attractants (If the feed is readily accepted by fish is called palatability-by attractive smell-Sugar cane molasses, amino acids, fatty acids, etc.)

4.3.4. Anti-nutritional factors (ANF)

The chemical components present in the feed ingredients will initiate the feeding behaviour of fish. For eg. in barley based feed if b-glucon (ANF) is present, it will affect the feeding behaviour of fish, therefore we must add the enzyme b-glucanase while preparing the feed, this will enhance the enzyme action during digestion of feed in fish. Similarly groundnut oil cake and soymeal contain phytic acid or tanic acid (ANF), if these ingredients are used for preparing feed, simultaneously add enzymes like Phytase and tannase. Further if this problems are rectified, the inorganic pollutants like phosphorus, Co_2, and ammonia can be reduced in the aquatic system (Francis *et al*., 2001).

4.3.5. Feed related waste accumulation

An aqua feed contains the selected plant based and animal based ingredients. This prepared feed will be supplied to the fish in the culture pond. During feeding behaviour of fish, digestion and assimilation are taking place, in turn the undigested materials, feed waste and faecal matter of fish will remain there. These materials are considered as solid and soluble waste. These are the feed related nutrients, contain all the nutritional sources like protein, carbohydrate, lipids and other food particles, which decompose and change the quality of water, lead to formation of inorganic pollution like phosphorus, Co_2, ammonia etc, and also lead to microbial growth (Fig.2 and Tables 8 & 9).

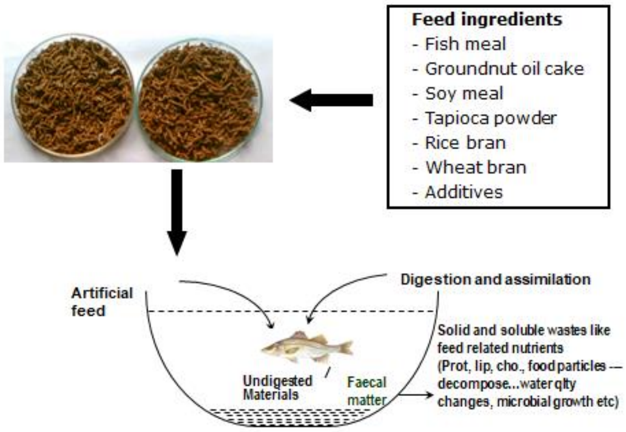


Fig.2. Feed related waste accumulation

Table 8. Relationship of FCR on solid and soluble waste accumulation in an aquaculture pond with the production rate of 2000 kg/Ha/yr.

FCR	Waste accumulation			
	Dry matter (kg)	Protein (kg)	Carbohydrate (kg)	Lipid (kg)
2 : 1 (4000 kg)	680	600	720	720
1.5 : 1 (3000 kg)	510	450	540	540
1.2 : 1 (2400 kg)	408	360	433	433

Note: Calculation of waste accumulation is based on the optimum digestibility of organic constituents by fishes

i. Dry matter digestibility = 83%

ii. Protein digestibility = 85%

iii. Carbohydrate digestibility = 82%

iv. Lipid digestibility = 82%

Table 9. Relationship of digestibility of organic constituents on waste accumulation in an aquaculture pond with the production rate of 2000 kg/ha/y

FCR	Protein		Carbohydrate		Lipid	
	Dig. (%)	Waste acc. (kg)	Dig. (%)	Waste acc. (%)	Dig. (%)	Waste acc. (kg)
1.5 : 1 (1500 kg)	80	300	80	300	78	330
1.5 : 1 (1500 kg)	90	150 (50%)	85	225 (25%)	85	225 (25%)
1.5 : 1 (1500 kg)	95	75 (75%)	90	150 (50%)	90	150 (50%)

Values in parentheses shows percentage reduction

5. Eco-friendly feed formulation

During feed formulation, we directly use the organic waste materials either from plant or animal source. The nutrients present in these materials sometimes not directly accepted by the fishes, because these materials will be in complex form and also not easily digested by the fish. Therefore we go for bioconversion process.

Bioconversion Process: Conversion of organic waste materials rich in nutrients into more acceptable forms by means of microbial process is called bioconversion (Joshi *et al*., 2021). During this process, the complex organic molecules are converted into simple monomeric units by specific microbes. Here microbes play an important role of conversion process with the help of certain enzyme sources produced by the microbes, these beneficial microbes are otherwise called as probiotic microbes. The resultant products is easily digested and assimilated by the candidate species either fish or prawn for their better performance. Few examples on bioconversion process are given below

5.1. Probiotics in bioconversion of prawn head waste

Prawn head waste contain rich amount of chitin, this chitin is converted into n-acetyl glucosamine by using chitin degrading microbes (chitinolytic microbes). The chitinolytic microbes includes bacteria (*Bacillus* sp., *Pseudomonas* sp., *Flavobacterium* sp., etc) and fungal species (*Aspergillus* sp., *Mucor* sp., *Mortierella* sp., etc). These chitin degrading microbes produce chitinase or chitobiase enzymes, which will degrade chitin by catalysing b-1,4 glucosidic bonds in low digestible chitin polymers and which will be turned into low molecular n-acetyl glucosamine which is a easily digestible product. Two enzymes mainly involved in this process are (i) Chitinase: Hydrolyses of chitin to n-acetyl-glucosamine as the end product and (ii) Chitobiase: Hydrolyses of chitobiose and chitotriose. Also the bioconversion of chitin is measured by analyzing n-acetyl-glucosamine liberated during fermentation process due to bacterial chitinolytic activity (Fig.3).

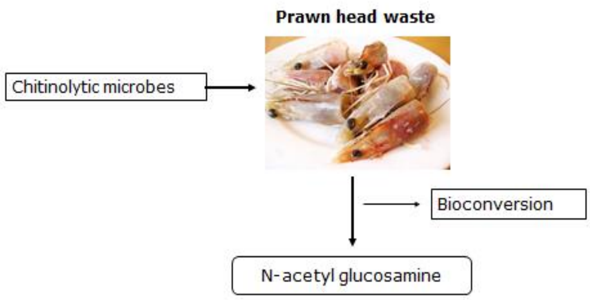


Fig. 3. Process of bioconversion of prawn head waste

5.2. Probiotics in bioconversion of Chicken intestine waste

Chicken intestine waste contain rich amount of fat and protein contents, these nutrients will be digested with the help of lipolytic and proteolytic microbes. The lipolytic bacteria such as *Alteromonas, Flavobacterium, Micrococcus, Stephylococcus, Psedomonas* spp, etc. and the fungal strains like *Aspergillus, Pencillium, Cladosporium* spp, etc. will produce lipase enzyme, which will cause lipolytic process by break down of lipid or fat into fatty acids. Similarly for proteolytic process, *Pseudomonas, Bacillus, Clostridium, Proteus, E.coli, Micrococcus* spp and proteolytic yeast species will produce proteolytic enzymes (Pepsin, trypsin, serine, cysteine, etc.). They digest the protein molecules or cleavage the protein through hydrolysis of peptide bonds into smaller peptides or aminoacids (Fig. 4 & 5)

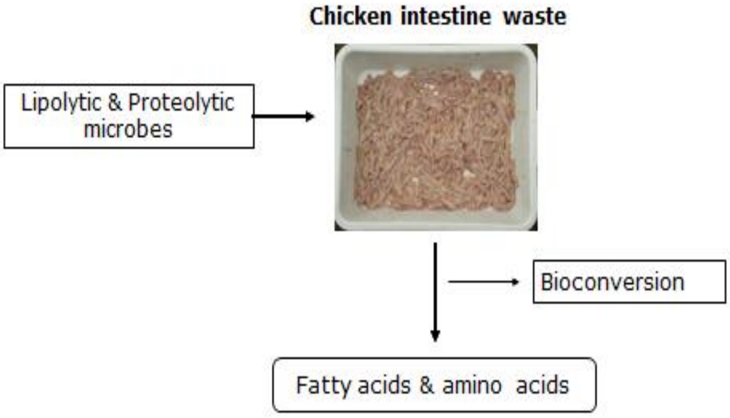


Fig. 4. Process of bioconversion of chicken intestine waste

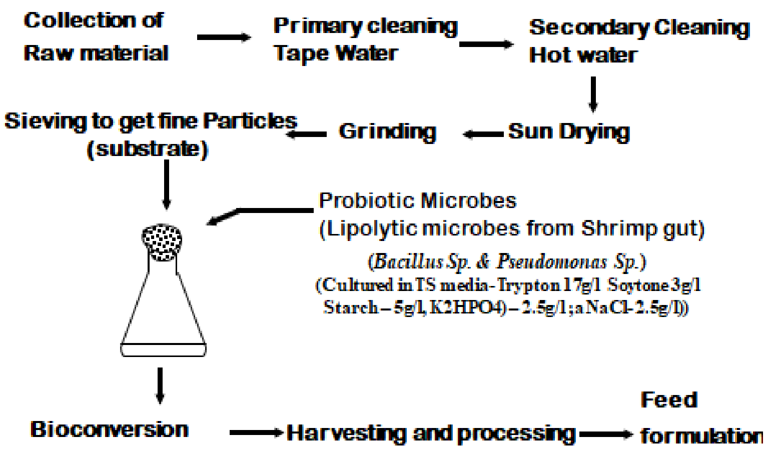


Fig.5. Phases involved in bioconversion of chicken intestine waste

5.3. Bioconversion of Sugarcane bagasse

Sugarcane bagasse contain high amount of cellulose, which will be converted to form reducing sugar with the help of cellulolytic microes (*Clostridium, Actinomycetes, Psedomonas, Acetobacter, Alcaligens* spp., etc). They produce certain enzymes like endoglucanase, b-D glucosidase and exocellobiohydrolase, which will degrade the cellulose to produce reducing sugar (Fig.6)

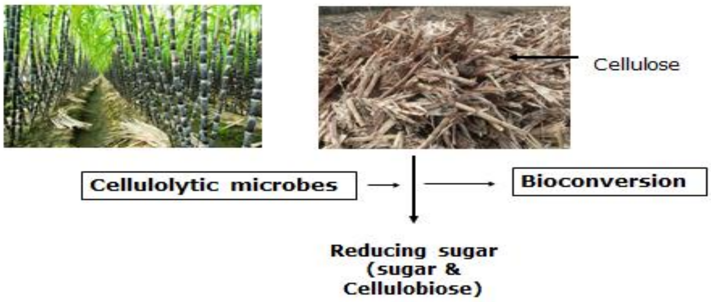


Fig. 6. Process of bioconversion of sugarcane bagasse

Cellulase enzyme system consist of three different enzyme and their role involve (i). Exocellobiohydrolase: Remove small amount of glucose and cellobiose from non-reducing end of cellulose
(ii). Endo 1-4 β-glucanase: Hydrolyses cellulose chain at random (Rapid changes in degree of polymerization)
(iii).b-glucosidase or cellobiase: It completes hydrolysis of cellobiose (cello-oligosaccharide strictly a cellulose)

Further bioconversion of cellulose can be measured by estimating reducing sugar by dinitrosalicylic acid method

5.4. Exoenzymes

Enzyme source added externally through artificial feed as additives in order to accelerate the process of digestion in candidate species is called exoenzymes (Ogunkoya *et al*., 2006)**.**

5.4.1. Production of microbial enzymes (exoenzymes) as feed additives

Microbial enzymes such as protease, amylase, pectinase etc. are called as probiotic enzymes, these enzymes specifically produced by certain microbes. These microbes are selected and inoculated into specific media with or without definite substrate and allow to ferment for 2-3 days. Here the microbes will synthesize two different mode of enzymes namely endo-enzyme or exo-enzymes. The endo-enzymes produce inside the microbial cells, further the cells are lysed to obtain this enzyme. Whereas exo-enzymes, synthesized by microbes and release into the culture medium, then by centrifugation process the exo-enzymes are obtained. The enzyme produced in both the ways are in crude form, further the enzyme may be purified (Fig.7).

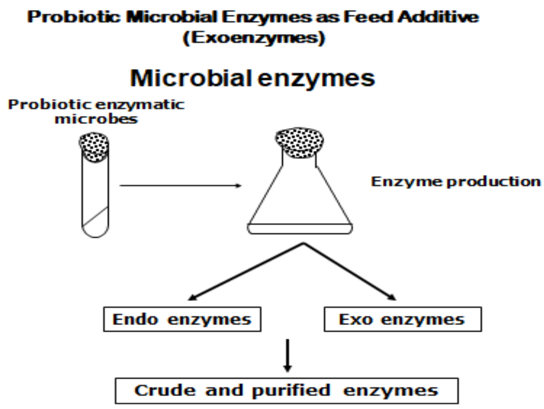


Fig.7. Probiotic microbial enzyme production

5.4.2. Uses of Exo-enzymes

- Enhance overall digestibility of feed and thereby improve feed efficiency
- Antagonize or destroy anti-nutritional factors (by specific enzymes) [Barley based feed - b-glucon (ANF) - b-glucanase (EN)] [Ground nut oil cake / Soy meal – Phytic acid (ANF) – Phytase (EN)]
- Render certain nutrients biologically available through increased digestibility and reduce its impact on environmental pollution (improve the availability of phosphorus in feed)

Before using these enzymes, we must know the optimum conditions required for better activation of the particular enzyme used. They are by knowing its optimum pH, temperature, and substrate concentrations for better activity and these must be monitored.

5.4.3. Incorporation of exo-enzyme in feed

Before using the enzyme, it must be aware of (i) choice of the enzyme to be incorporated (What enzyme to be used?), (ii) action of the enzyme should be clearly understood, (iii) stability of the enzyme, especially in the digestive tract of fish along with the physiological conditions to the enzyme activity in the digestive tract and (iv) stability of the enzyme during feed pelleting process. Prompted method of adding exo-enzymes in liquid form during post pelleting process is an important one. Heat stable commercial enzyme preparations (protease, lipase, amylase, hemicellulase) are available, which can be readily used, for eg. Aqua-zymes, a commercial product, it is stable at 85 - 105^{0}C for several minutes and loss of enzyme activity is negligible during pelleting processes. Polyzyme (combination of enzyme such as amylase, protease, b-glutanase, cellulose and xylanase), it is available in the name of Superzyme CS, produced by Canada Biosystem Inc., Alberta, Canada. This product is emulsified with fish oil and top coated on the pellet feed and fed to rainbow trout (*Oncorhyncus mykiss*) and observed good digestibility result and growth performance (Ogunkoya *et al*., 2006). Some of the important exo-enzymes incorporated in the fish or prawn feed is given in Table (10)

Table 10. Exo-enzymes used in fish and prawn feeds

Enzyme / source	Species	Effect on performance parameters	
		FCR	SGR
Lacto-Sacc (commercial, India) (2.5g/kg)	*Penaeus indicus*	1.70 (3.28)	3.03 (2.01)
Fungal protease (1%) (Commercial, USA)	*Liptopenaeus vannamei*	2.3 (2.6)	2.5 (2.2)
Multi-zyme AK (1%) (Commercial, USA)	*Liptopenaeus vannamei*	2.5 (2.6)	2.3 (2.2)
Enzeco protease (1%) (Commercial, USA)	*Liptopenaeus vannamei*	2.6 (2.6)	2.3 (2.2)
Cenzyme (1%) (Commercial, USA)	*Liptopenaeus vannamei*	2.5 (2.6)	2.3 (2.2)
Polyzymes* Commercial (1.5 kg/tonne)	*Cyprinus carpio*	Significant influence	
Pepain (0.8%) (Commercial, Germany) 25% protein diet	*Cyprinus carpio*	2.78 (3.04)	2.39 (1.99)
30% protein diet		2.30 (2.55)	2.23 (1.96)
35% protein diet		1.78 (1.84)	2.99 (2.44)
40% protein diet		1.41 (1.55)	2.98 (2.61)

* Polyzymes: Stabilized mixture of Amylase, Protease, β-gluconase, β-glucosidase & cellulose

NB: Values in the parenthesis is the control (Devoid of enzyme)

6. Probiotics as feed additives

Live beneficial microbial cells either in broth/pellet form or lyophilised powder form can be incorporated in the feed directly as feed additive, to enhance the digestibility capacity of fish and also to enhance the growth of the fish. In some cases, the probiotic cells are added in the culture medium to control the proliferation of disease causing pathogenic microbes.

The other benefits of probiotic feed additives includes

(i). Adhesion and colonization of gut

(ii). Increase population of useful gut microflora (iii). Produce vitamins, enzymes or antibiotics in the gut and

(iv). Reduce the levels of toxic amines and ammonia in the gut

- Quality of probiotics depends upon storage conditions since they are either live microbes or their fermentation products.
- Viability, strain differences, dosage and frequency of feeding also determine quality and usefulness of probiotics.

6.1. Probiotic interaction in the gut of fish

The efficiency of probiotic microbes mainly depends on its colonization and proliferation in the gut of the host fish. The microflora forms an ecosystem with the host, comprising of (i) Biotic components (indigenous and transient microbes & gastro intestinal epithelial cells which delimit the biotics, (ii) Abiotic components (Dietary origin & undigested materials) and (iii) Endogenous components (from saliva, gastric, pancreatic and intestinal secretions or excretions such as enzymes, hormones, mucus, bile salt, urea, immunoglobulins, peptides and several other components (Manpal Sridhar, 1998). If all these components interact well, i.e., the interaction is compactable, the proliferation of the microbes will be at a faster rate and the host will have beneficial effect

6.2. Metabolic interaction of probiotics in the gut

It includes the suppression of reactions which result in the generation of toxic and carcinogenic metabolites. By stimulating the enzyme reactions involved in detoxification of potentially toxic substances either ingested or produced endogenously. By stimulating enzymes involved in the digestion of complex nutrients or providing a bacterial source of enzyme; where such enzymes are absent due to genetic disorders or disease. By synthesizing vitamins and other essential nutrients which are not provided in sufficient quantities in the diet.

6.3. Factors affecting the probiotic response in the host

The interaction of microbial feed supplement with host animal and its gut microflora is very complex due to composition of the host animal gut flora, dosage region which may be continuous or as a single dose, age and species of fish (physiology and immune status may vary), nutritional status of the host gut, quality assurance (which is viability of the probiotic product and strain variation) and production method of the probiotic product may cause variation in response for the same species

6.4. Evaluation of probiotic bacteria in laboratory condition

In the laboratory, a probiotic bacterium was isolated from the gut of shrimp *P. monodon.* The antagonistic activity of this probiotic strain was tested against shrimp pathogens and found good result. Then screened this probiotic strain for production of enzymes such as protease, amylase, lipase and phytase. Then through molecular (16 SrRNA) analysis, the strain was identified as *Bacillus cereus*. The strain was mass cultivated and subsequently lyophilised the cells. Prepared low cost probiotic incorporated diet by supplementing this lyophilised cells (@ 400mg cells/100 g feed) in one set (A feed) and another set with low cost probiotic product (@ 4% cells in enriched culture mixed in commercial talc powder- B feed) supplemented feed. Then two separate feeding experiments in shrimp *P. monodon* were conducted for 90 days in outdoor culture system by providing the respective feeds. At the end of the experiment, i.e. 90th day, the survival and growth response as well as immunological parameters were analysed separately for both A and B diets fed shrimp and compared the results (Naveen *et al*., 2016) (Fig.8).

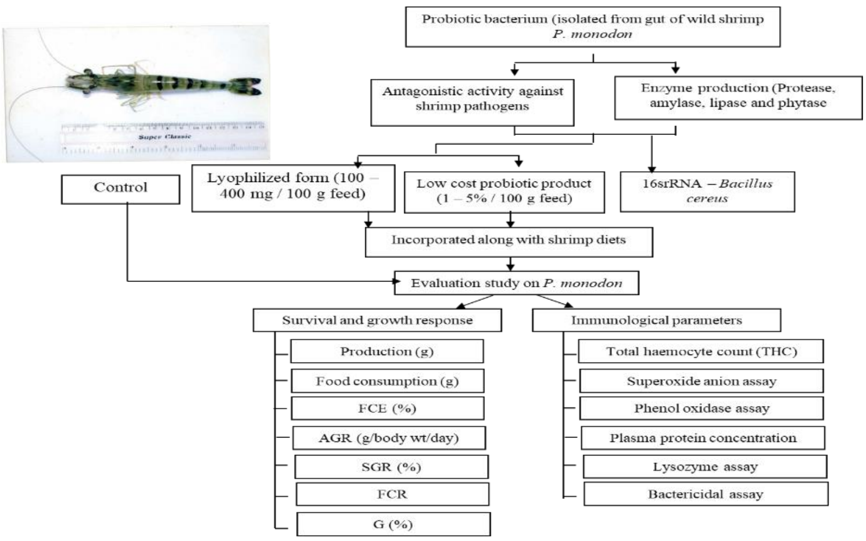


Fig. 8. Evaluation of a probiotic bacterium for growth and immune response in shrimp *P.monodon* (a schematic process)

The results observed that the Specific growth rate (SGR) of feed A and feed B fed shrimp showed more than 19.88 and 8.40 % respectively when compared with control diet fed shrimp, which devoid of probiotics. Similarly the food conversion rate (FCR) was observed as more than 34.53 and 30.72 % respectively in A and B diet fed shrimp, when compared with the control diet fed shrimp. The nonspecific immune parameters such as Lysozyme content (> 47.04 & >40.0% in A & B diet than control), pro Phenol oxidase content ((> 24.07 & >55.29% in A & B diet than control), super oxide anion assay (> 32.71 & 54.79% in A & B diet than control) and Bactericidal activity (> 16.0 & >17.0% in A & B diet than control) were improved much in both probiotic supplemented diets fed shrimp.

7. Conclusion

The present report explained the details about fish feed formulation and production through feeding strategies, types of aquaculture feeds, quality maintenance of feeds, and feed production through bioconversion process. The formulated diets are good source of required nutrients for cultivable fish; therefore it is necessary to prepare the suitable eco-friendly feed by following appropriate techniques by utilising available resources. Aquaculture sustainability will still depend in the foreseen future on supply of quality fish feeds and it could be considered as one of the inevitable components for the vitality of aquaculture industry.

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Chapter: 8

Addressing the Challenges in Aquaculture Nutrition Amid Difficult Climatic Conditions

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Abstract

Aquaculture is a critical component of global food production, yet its expansion is increasingly hampered by the impacts of climate change on essential water quality parameters, which are vital for the health and nutrition of aquatic organisms. This paper explores the manifold effects of adverse climatic conditions on aquaculture nutrition and presents innovative feed formulation approaches to mitigate these challenges. It probes into the impact of climate change on the availability and costs of aquaculture feed ingredients, offering strategies to address these issues through advanced feed formulation techniques. Additionally, the paper outlines future directions to ensure the sustainability and resilience of aquaculture amidst climate change, focusing on feed formulation perspectives.

Keywords: Aquaculture nutrition, climate change, feed formulation, fish health, sustainable aquaculture.

1. Introduction

Aquaculture has experienced significant growth in recent decades, becoming a vital component in meeting the world's increasing demand for seafood as wild fish stocks decline. Beyond its role in food production, aquaculture offers economic opportunities and enhances food security for many communities. However, this industry faces substantial challenges due to climate change. Human activities have largely driven climate change, profoundly altering the environments where aquatic organisms live. Rising global temperatures, changing rainfall patterns, ocean acidification, and an increase in the frequency of extreme weather events such as storms and heatwaves (Barange *et al*., 2018) are disrupting aquatic ecosystems and posing significant challenges to aquaculture operations worldwide. These environmental shifts impact various aspects of aquatic life, including habitats, growth rates, reproductive abilities, and susceptibility to diseases

(Reid *et al*., 2019). Consequently, there is an urgent need to adapt aquaculture practices, particularly in managing nutrition and feeding regimes for these organisms. The Food and Agriculture Organization (FAO) emphasizes the importance of enhancing the resilience of aquaculture to withstand these climate impacts, ensuring sustainable food production (Stankus *et al*., 2021).

This paper explores how climate change affects aquaculture nutrition and discusses mitigation strategies through innovative feed formulation approaches. Additionally, it examines the impact of climate change on the availability and costs of aquaculture feed ingredients and presents remedial strategies. Future research directions are also outlined to promote sustainable aquaculture in a changing climate.

2. Impacts of Adverse Climatic Conditions on Aquaculture Nutrition Thorough Alterations in Water Quality Parameters

Water quality is a crucial factor in aquaculture, directly influencing the health, growth, and nutrient utilization of aquatic organisms. Climate change significantly impacts several key water quality parameters, including temperature, pH, dissolved oxygen (DO), and salinity. Maintaining these parameters within optimal ranges is essential for effective aquaculture practices.

2.1 Temperature Changes

Temperature is a critical environmental factor in aquaculture because it directly affects the metabolic rates of aquatic organisms. As temperatures rise due to climate change, aquatic habitats experience fluctuations that can exceed the optimal ranges for many species. This thermal stress alters the physiology and behavior of aquatic animals, leading to significant effects on their feeding patterns and nutritional requirements (Cascarano *et al*., 2021).

Elevated temperatures generally accelerate metabolic activities in fish and other aquatic organisms. This increased metabolism demands higher energy expenditure, resulting in higher feed intake to meet the energy requirements of the animals (Mugwanya *et a*l., 2022). However, while increased metabolism can initially lead to higher feed consumption, prolonged exposure to high temperatures can reduce the efficiency with which animals convert feed into body mass, known as reduced feed conversion efficiency (RFE) (Alfonso *et al*., 2021). Fish may require more feed to maintain their growth rates under such thermal stress conditions, leading to increased operational costs for aquaculture operations.

Aquatic animals often adjust their feeding behavior in response to temperature changes. Higher temperatures can increase appetite and feeding frequency as fish seek to meet their increased metabolic demands. However, extreme temperatures that exceed optimal ranges can suppress appetite, reducing feed intake and growth rates (Handeland *et al*., 2008).

Temperature also affects digestive enzyme activity and nutrient absorption in fish. Optimal temperatures enhance the efficiency of digestive enzymes, facilitating nutrient absorption from feed. However, under high-temperature stress, digestive enzyme activity can decrease, reducing nutrient uptake and potentially leading to nutrient deficiencies (Volkoff & Rønnestad, 2020).

Furthermore, temperature stress compromises the immune function of aquatic animals. High temperatures can weaken the immune response, making fish more susceptible to diseases and infections. This vulnerability is exacerbated by the physiological adjustments that fish undergo to cope with thermal stress, diverting energy away from immune defense mechanisms (Franke *et al*., 2024). Warmer temperatures can also accelerate the lifecycle of pathogens and parasites, increasing their prevalence in aquaculture systems (Cascarano *et al*., 2021). Disease outbreaks not only affect the health and welfare of farmed fish but also result in economic losses for aquaculture operations due to increased treatment costs.

2.2 pH Changes

Changes in pH levels due to climate change can significantly affect nutrient availability and enzymatic activities in aquatic organisms. pH directly influences the solubility and availability of essential minerals and nutrients in water, which are critical for growth and metabolic processes in fish and other aquatic species (Niinemets *et al*., 2017). Variations in pH can alter the chemical form and availability of nutrients, affecting their uptake and utilization by aquatic organisms.

In acidic or alkaline conditions, essential nutrients such as phosphorus, calcium, and magnesium may become less available to aquatic organisms. This limitation can impact metabolic processes, skeletal development, and overall growth rates in fish (Lall & Kaushik, 2021).

pH levels also influence enzymatic activities involved in digestion and nutrient assimilation. Optimal pH conditions are necessary for the proper function of digestive enzymes, ensuring efficient breakdown of feed components and absorption of nutrients (Pavasovic *et al.*, 2004). Deviations from optimal pH levels can impair these enzymatic functions, leading to reduced feed efficiency and potential nutrient deficiencies.

2.3 Dissolved Oxygen (DO) Fluctuations

Dissolved oxygen (DO) is critical for aerobic respiration in aquatic organisms, supporting their metabolic activities and growth. Climate change exacerbates factors that can lead to fluctuations in DO levels within aquaculture systems. Warmer water temperatures, a consequence of climate change, reduce the solubility of oxygen in water. This thermal effect decreases the amount of oxygen available to fish and other aquatic organisms, particularly in aquaculture settings where water may be artificially heated or naturally warmer due to environmental changes (Gallo *et al.*, 2020).

Increased nutrient inputs from agricultural runoff or aquaculture effluents can lead to eutrophication, promoting algal blooms. These blooms can deplete DO levels through increased microbial respiration and decomposition processes, creating hypoxic or anoxic conditions detrimental to aquatic life (Wurtsbaugh *et al.*, 2019). Reduced DO levels impair aerobic metabolism in fish, limiting their ability to efficiently utilize nutrients from feed. This oxygen deficit can lead to slower growth rates, decreased feed conversion efficiency, and increased susceptibility to stress and diseases (Glencross, 2009).

Increased nutrient inputs from agricultural runoff or aquaculture effluents can lead to eutrophication, promoting algal blooms. These blooms can deplete DO levels through increased microbial respiration and decomposition processes, creating hypoxic or anoxic conditions detrimental to aquatic life (Wurtsbaugh *et al.*, 2019). Reduced DO levels impair aerobic metabolism in fish, limiting their ability to efficiently utilize nutrients from feed. This oxygen deficit can lead to slower growth rates, decreased feed conversion efficiency, and increased susceptibility to stress and diseases (Glencross, 2009).

2.4 Salinity Changes

Changes in salinity levels, influenced by climate-induced factors such as sea-level rise and variations in freshwater inputs, have significant implications for osmoregulation and nutrient uptake in aquatic organisms. Marine and estuarine species are adapted to specific salinity ranges, and deviations from these optimal conditions can stress physiological processes such as osmoregulation. Fluctuating salinity levels challenge fish to maintain internal ion concentrations, affecting metabolic functions and overall health (Podbielski *et al*., 2022).

Salinity fluctuations can also impact the availability and uptake of essential nutrients. Changes in water salinity alter the permeability of fish gills and digestive membranes, influencing the absorption of nutrients from feed. This disruption can affect growth rates, reproductive success, and immune responses in aquatic organisms (Ruiz-Jarabo *et al*., 2015).

3. Mitigating the Effects of Adverse Climatic Conditions on Aquaculture Nutrition Caused Due to Alterations in Water Quality Parameters

Climate change-induced alterations in water quality parameters such as temperature, pH, dissolved oxygen (DO), and salinity significantly impact the health, growth, and nutritional efficiency of aquatic organisms. Adapting feed formulation strategies to mitigate these effects is essential for sustaining aquaculture productivity. Some scientific approaches to address these challenges through innovative feed formulations are highlighted below:

3.1 Addressing Temperature Variations Through Feed Formulation

Enhanced Energy-Dense Feeds: As temperatures rise, the metabolic rate of aquatic organisms increases, demanding higher energy intake. Formulating energy-dense feeds can help meet these increased metabolic needs. Feeds enriched with lipids and easily digestible carbohydrates provide the necessary energy boost without overloading the protein content, which can otherwise increase nitrogenous waste and affect water quality (Glencross *et al*., 2020).

Heat-Stable Nutrients and Enzymes: High temperatures can degrade certain nutrients and reduce the activity of digestive enzymes. Incorporating heat-stable vitamins and minerals, as well as using enzyme supplements that retain activity at elevated temperatures, can enhance nutrient absorption and overall feed efficiency (Eriegha *et al*., 2017).

Antioxidant-Rich Diets: Thermal stress increases oxidative stress in fish. Adding antioxidants such as vitamins C and E, selenium, and carotenoids to the diet can protect cellular components from oxidative damage, improving fish health and growth under high-temperature conditions (Trenzado *et al*., 2006).

3.2 Addressing pH Fluctuations Through Feed Formulation

pH-Buffering Feed Additives: Feed formulations can include pH-buffering agents like sodium bicarbonate and calcium carbonate to stabilize the gut pH, ensuring optimal conditions for digestive enzyme activity and nutrient absorption. This approach helps mitigate the adverse effects of fluctuating external pH on internal digestive processes (Lall & Kaushik, 2021).

Chelated Minerals: To counteract the reduced availability of essential minerals in acidic or alkaline conditions, incorporating chelated minerals (minerals bound to organic molecules) can enhance their bioavailability. Chelated forms of minerals such as zinc, iron, and copper are more readily absorbed and utilized by aquatic organisms (Jobling, 2012).

3.3 Addressing DO Fluctuations Through Feed Formulation

Oxygen-Releasing Compounds: In environments with low DO levels, incorporating oxygen-releasing compounds such as peroxides or other oxygen donors in feed can help alleviate hypoxia. These compounds gradually release oxygen, improving aerobic respiration and nutrient utilization (Chowdhury & Fotedar, 2006).

High-Quality Proteins: High-quality protein sources with balanced amino acid profiles can enhance feed conversion efficiency under low DO conditions. Proteins from fishmeal, soybean meal, and other plant-based sources should be optimized to ensure minimal metabolic waste and efficient nutrient uptake (Gatlin *et al*., 2007).

3.4 Addressing Salinity Fluctuations Through Feed Formulation

Osmoregulatory Supplements: Feeding strategies can include osmoregulatory supplements like betaine and taurine, which support cellular osmoregulation and ion balance in varying salinity conditions. These supplements help maintain osmotic balance and reduce the energy expenditure required for osmoregulation (Salze *et al*., 2012).

Balanced Electrolytes: Ensuring the diet contains balanced levels of electrolytes such as sodium, potassium, and chloride supports the maintenance of ion balance across cell membranes, which is critical under fluctuating salinity conditions. Proper electrolyte balance aids in osmoregulation and overall physiological stability (NRC, 2011).

4. Some Studies Addressing Water Quality Challenges via Innovative Feed Formulation

This section discusses few interesting studies that showcase effective strategies in mitigating the impacts of climate change on aquaculture through careful formulation of feeds and adaptive practices.

Due to prolonged heatwaves causing reduced fish growth rates and increased disease susceptibility among farmers, researchers developed an energy-dense feed enriched with lipids and antioxidants. This formulation aimed to meet increased metabolic demands and counteract oxidative stress. Fish oil and soy lecithin were included to boost caloric content, while vitamins C and E, alongside selenium, were incorporated to mitigate oxidative stress. The adjusted feed significantly improved growth rates during heatwaves compared to standard feeds, leading to a notable decrease in mortality rates from heat-induced stress and related diseases (Trenzado *et al*., 2006; Glencross *et al.*, 2007).

In the other study, researchers adopted a feed formulation that included pH-buffering agents and chelated minerals to stabilize gut pH and enhance nutrient absorption of a shrimp exposed to fluctuating pH levels in pond water. Sodium bicarbonate and calcium carbonate were added to the feed. Chelated forms of zinc and iron were included to improve bioavailability. Shrimp growth rates and survival rates improved significantly compared to control groups. The buffering agents helped maintain optimal gut pH, leading to better nutrient absorption and overall health (Pavasovic *et al*., 2004; Lall & Kaushik., 2021).

In another notable study, researchers developed a precision nutrition approach to optimize feed formulations according to the specific requirements of fish across different life stages. This strategy involved incorporating alternative protein sources to tailor feeds, such as lupin and insect meal, thereby reducing dependency on traditional fishmeal and soybean meal. Additionally, algal and microbial proteins were integrated to diversify protein sources and mitigate vulnerability to supply fluctuations. As a result, there was a substantial improvement in feed conversion efficiency and a significant reduction in overall feed costs. The adoption of alternative proteins also contributed to lowering the environmental impact of aquaculture operations (NRC, 2011; Henry *et al*., 2015).

5. Impact of Climate Change on Aquaculture Feed Ingredient Availability and Costs

Climate change has a profound impact on global ecosystems and economies, and the aquaculture sector is no different. One crucial aspect affected by climate change is the availability and cost of ingredients used in aquaculture feeds, which in turn affects the stability of feed supply.

5.1 Climate Change Impacts on Agricultural Productivity

Climate change alters temperature patterns, significantly affecting crop yields, which are crucial sources of plant-based feed ingredients like soybean meal, corn, and wheat. Higher temperatures can induce heat stress, reducing the efficiency of photosynthesis and crop growth rates (Lobell *et al*., 2011). Moreover, extreme heat can exacerbate evapotranspiration rates, leading to water deficits that further stress crops.

Changes in precipitation patterns, including increased frequency and intensity of droughts and floods, disrupt optimal growing conditions for crops. Drought conditions reduce soil moisture availability, while excessive rainfall and flooding can cause root anoxia and soil erosion, both detrimental to crop productivity (Lesk *et al*., 2016). These disturbances can result in reduced yields and greater variability in crop production, impacting the availability and cost of plant-based feed ingredients.

5.2 Climate Change Impacts on Marine Resources

Climate change impacts on marine resources are significant, especially concerning marine ingredients like fishmeal and fish oil essential for aquaculture feeds. Ocean warming, a consequence of climate change disrupts the distribution, abundance, and reproductive cycles of marine species. Many fish species are shifting towards cooler waters, altering traditional fishing grounds and potentially reducing the availability of fish stocks used for fishmeal and fish oil production (Cheung *et al*., 2013).

Furthermore, increased CO2 levels contribute to ocean acidification, which adversely affects the health of marine ecosystems. Shellfish and other calcifying organisms, crucial in marine food webs are particularly vulnerable to these changes. The decline in these populations can have cascading effects on the availability of marine resources utilized in aquaculture feeds (Kroeker *et al*., 2013).

5.3 Climate Change Impacts on Supply Chains

Climate change-induced variability in crop and fish production contributes to fluctuations in global markets. As supply becomes more unpredictable, prices of feed ingredients become more unstable. This economic uncertainty can lead to higher costs for aquaculture operations, impacting the profitability and sustainability (Nelson *et al*., 2014).

Additionally, climate-induced variability in crop and fish production contributes to fluctuations in global markets. This unpredictability in supply leads to instability in feed ingredient prices. The resulting economic uncertainty can elevate costs for aquaculture operations, impacting their profitability and long-term sustainability (Nelson *et al*., 2014).

6. Adaptive Strategies for Mitigating Climate Change Impacts on Aquaculture Feed Ingredient Availability and Cost

Climate change significantly disrupts the availability and cost of aquaculture feed ingredients by affecting agricultural productivity, marine resource availability, and supply chain stability. These disruptions pose challenges to the sustainability and profitability of the aquaculture industry. However, by adopting adaptive strategies such as diversifying feed ingredients, enhancing feed efficiency, promoting sustainable farming practices, and implementing supportive policies, the industry can mitigate these impacts and ensure a stable feed supply.

Diversification of Feed Ingredients: One adaptive strategy is to diversify the sources of feed ingredients. Utilizing a broader range of plant-based proteins, such as lupins, peas, and insect meals, can reduce dependency on any single crop and mitigate the risk of supply disruptions (Henry *et al*., 2015). Research and development into alternative feed ingredients, including algae and microbial proteins, can also provide sustainable and resilient options.

Enhanced Feed Efficiency: Improving feed efficiency through advancements in feed formulation and technology can help mitigate the impacts of climate change on feed supply. The use of precision nutrition, which tailors feed composition to the specific needs of different fish species and life stages, can optimize feed utilization and reduce waste (NRC, 2011).

Sustainable Farming Practices: Adopting sustainable farming practices that enhance soil health and water use efficiency can help stabilize crop yields. Techniques such as conservation agriculture, agroforestry, and integrated pest management can improve the resilience of crop production systems to climate variability (Smith *et al*., 2013).

Policy and Economic Measures: Implementing policies that support sustainable aquaculture and agriculture can play a crucial role in mitigating climate impacts. Subsidies for sustainable practices, investment in climate-resilient infrastructure, and international cooperation to stabilize global markets are essential measures to ensure feed supply stability (FAO, 2018).

7. Few Studies Mitigating Climate Change Effects on Aquaculture Feed Ingredients

Several studies highlight how innovative feed formulation strategies, such as incorporating alternative proteins, algae, and agro-industrial by-products, effectively tackle the challenges posed by climate change on aquaculture feed ingredient availability and cost. Embracing these strategies improves feed supply stability, lowers feed costs, and strengthens the resilience of aquaculture operations against climate variability.

Due to climate-induced fluctuations threatening the aquaculture industry in many countries, researchers have formulated feeds using locally available and sustainable alternative ingredients. They replaced a portion of traditional feed ingredients like soybean meal and corn with local crops such as cassava and rice bran. Additionally, black soldier fly larvae meal was introduced as a protein source to reduce dependence on fishmeal. This new feed formulation maintained growth performance and feed conversion ratios comparable to traditional feeds. Furthermore, it significantly reduced feed costs and decreased reliance on imported soybean meal and fishmeal (Kumar *et al*., 2012; Henry *et al*., 2015).

In another study, researchers incorporated algae as an alternative feed ingredient, utilizing both microalgae and macroalgae to supply essential proteins and omega-3 fatty acids in place of traditional fishmeal and fish oil. This inclusion of algae in fish feed resulted in similar growth rates and enhanced omega-3 content in the fish. Moreover, it significantly reduced reliance on fishmeal and fish oil, leading to decreased feed costs (Tacon *et al*., 2015; Campbell & Glencross, 2018).

In another notable study, researchers explored the use of agro-industrial by-products in shrimp feed formulations. They substituted corn and soybean meal with rice bran and wheat middlings, while incorporating fish offal and other processing waste as protein sources to reduce reliance on conventional fishmeal. This innovative feed formulation sustained shrimp growth performance and enhanced feed conversion ratios. Additionally, it significantly reduced feed costs and stabilized ingredient supply through the utilization of agro-industrial by-products (Hardy & Barrows, 2014; Rani *et al*., 2018).

8. Future Directions

To ensure the sustainability and resilience of aquaculture in the face of climate change, future research and practices should focus on the following areas:

Development of Climate-Resilient Feed Ingredients: Future research should prioritize the development of new feed ingredients that are less susceptible to climate-induced fluctuations. This includes exploring underutilized crops, novel proteins such as insect meal and single-cell proteins, and marine algae. These ingredients need thorough evaluation for their nutritional profiles, digestibility, and ecological impacts.

Precision Nutrition and Custom Feeds: There is a need for feed industries to adopt precision nutrition, tailoring feed formulations to meet the specific requirements of different aquaculture species and developmental stages. Advanced digital tools can optimize feed composition and minimize waste, integrating climate variables to ensure optimal nutrient uptake under varying environmental conditions.

Enhanced Feed Efficiency: Feed formulators must continue their efforts to improve feed conversion ratios through better feed formulations and the use of feed additives that enhance nutrient absorption and metabolism. This includes the development of heat-stable nutrients, probiotics, and prebiotics that support gut health and overall resilience of aquatic organisms.

Integrated Multi-Trophic Aquaculture (IMTA): Encouraging fish farmers to adopt IMTA systems, where multiple species with complementary feeding habits are cultivated together, can optimize nutrient utilization, reduce waste, and enhance the ecological balance of farming operations. This approach strengthens resilience to climate change.

Sustainable Sourcing and Circular Economy: Researchers should promote the use of sustainable feed ingredients sourced from agro-industrial by-products and waste materials. Implementing circular economy principles in aquaculture can decrease reliance on finite resources and minimize the environmental impact of feed production.

Policy and Economic Incentives: dvocating for policies that support sustainable aquaculture practices, such as subsidies for research on alternative feed ingredients and investments in climate-resilient infrastructure, is crucial. Economic incentives can facilitate the adoption of innovative farming practices and technologies by farmers.

Monitoring and Adaptive Management: Establishing robust monitoring systems to track climate variables and their effects on aquaculture operations is essential. Adaptive management strategies based on real-time data can help mitigate adverse impacts and optimize production in response to changing environmental conditions.

9. Conclusion

In conclusion, the aquaculture industry plays a pivotal role in global food security and economic prosperity, yet it confronts profound challenges exacerbated by climate change. Variations in temperature, pH, dissolved oxygen, and salinity directly impact the health, growth, and nutritional efficacy of farmed aquatic species. Moreover, climate-induced disruptions in feed ingredient availability and costs further jeopardize aquaculture sustainability. Addressing these challenges requires a heterogeneous approach that includes the development of energy-dense, heat stable nutrients and enzyme supplemented, antioxidant-rich feeds, the use of pH-buffering agents, the incorporation of pH buffering feed additives, and chelated minerals, and the incorporation of oxygen-releasing compounds with high quality proteins and osmoregulatory supplements in the feed.

Additionally, diversifying feed ingredients, improving feed efficiency through precision nutrition, adopting sustainable farming practices, and implementing policies that support sustainable aquaculture are essential strategies to ensure a stable feed supply in the future. By adopting these strategies, the aquaculture industry can enhance its resilience, ensure food security, and contribute to sustainable development in the face of climate change.

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Chapter: 9

Global Trends in Sustainable Feed Ingredients for Climate Resilient Aquaculture

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1. Introduction

Given the projected human population of 9.7 billion by 2050 (UN 2015), there is an anticipated need for a 25–75% increase in the production of nutrient-rich food to meet future demands (Hunter et al., 2017). The growing middle class in Africa and Asia has also led to a shift in dietary preferences towards animal-sourced proteins (Goodman & Robison, 2013; Tschirley et al., 2015). This increase in livestock production raises significant environmental concerns, including land conversion, overexploitation of grasslands, and greenhouse gas emissions (Michalk et al., 2018). As a result, aquaculture has emerged as a vital strategy for enhancing animal protein production and ensuring food security (Hua et al., 2019; FAO, 2020). Over the past three decades, aquaculture has been the fastest-growing food production sector, surpassing global beef production in 2012 (FAO, 2018; Larsen & Roney, 2013). It offers numerous benefits, such as poverty reduction in low-income countries (Filipski & Belton, 2018), increased production through innovative culture systems and genetic improvement technologies (Gratacap et al., 2019; Khanjani & Sharifinia, 2020; Azra et al., 2022), and the cultivation of eco-friendly species like silver carp and nutrient-extractive species such as seaweed (Poore & Nemecek, 2018; Chopin & Tacon, 2021).

As part of its 2030 development agenda, the United Nations unveiled 17 sustainable development goals (SDGs) in 2015 with the aim of fostering world peace and prosperity. Aquaculture is helping achieve these objectives more and more as it grows quickly; in particular, it is helping to conserve natural resources and cut waste by turning it into food for fish and crustaceans (Tacon & Metian, 2015). The utilization of resources and the environment to meet current demands without compromising the ability of future generations to meet their own needs is known as sustainable development. Social, economic, and environmental sustainability serve as its three foundations (World Commission on Environment and Development, 1987). By supplying aquatic nutrients for human use without endangering ecosystems or depleting natural resources, sustainable aquaculture seeks to strike a balance between these pillars (Boyd et al., 2020). However, because the interests of the pillars sometimes conflict, striking this equilibrium can be difficult. To further enhance sustainable aquaculture practices globally through integration, evidence, policy, and regulation, creative, multifaceted frameworks such as the One Health lens are being developed (Stentiford et al., 2020). The present and potential directions of sustainable aquaculture feed production are examined in this review.

2. Aquaculture Feed

Both "fed" species (such as shrimp, sea bass, and salmon) and "non-fed" species (such as silver carp, seaweed, and oysters) are produced via aquaculture . Historically, aquafeeds with high concentrations of fishmeal and fish oil (FMFO) from forage fish harvested in the wild have been a major source of protein, micronutrients, and enhanced palatability for fed aquaculture (Froehlich et al., 2018). Because it puts more strain on wild fish supplies that are already in decline and upsets aquatic food webs, the use of FMFO is seen to be the main unsustainable element in aquaculture (Ghamkhar & Hicks, 2020; Huang et al., 2019). Direct-fed species currently make up around 68% of the world's total aquaculture production of fish and crustaceans (Tacon, 2020).

According to Tacon and Metian (2015), this will cause the whole production of aquafeeds to increase by 75%, from 49.7 million tons in 2015 to 87.1 million tons by 2025. According to estimates, fed aquaculture will soon outgrow the natural supply of fodder fish if expansion continues at the current rate (Froehlich et al., 2018).

The buildup of chemicals, heavy metals, and microplastics in marine fish as a result of industrialization and urbanization is another problem with fishmeal taken from the wild (Hanachi et al., 2019). In spite of this, high-value fish and crustaceans are fed from about 12% of the total wild-caught fisheries (FAO, 2018), contributing to an average annual output growth rate of 5.70% for fish and 9.91% for crustaceans since 2000 (FAO, 2019). High-value feed species are cultivated in developing countries and exported to wealthy nations, driven by economic incentives (Hua et al., 2019). While fed aquaculture's dependence on existing aquafeeds is bad for the environment, it has been demonstrated to improve food security and reduce poverty in developing nations, where the majority of production takes place (Silva & Davy, 2010; Belton et al., 2018; Hoover et al., 2019).

Aquaculture must thus strike a balance between raising high-value fed species and low-input unfed species if it is to make a substantial contribution to future food security and financial alleviation.

Generally, 40–80% of the expenses associated with aquaculture production are related to feed (Rana et al., 2009; Ayadi et al., 2012; Okomoda et al., 2020). Small-scale producers and rural farmers are primarily impacted by these costs, which are rising owing to a number of causes (such as shortages of cereal crops, oil prices, global warming, and demand) (Rana et al., 2009). Global demand is predicted to drive up FMFO prices by as much as 13% by 2030 (FAO, 2020). The biggest obstacle to sustainability in the aquaculture industry is its continued reliance on FMFO (Tacon et al., 2022). Various raw materials have been adopted to reduce FMFO inclusion in aquafeeds due to the restrictions and environmental effect of meals supplied from forage fish, as well as rising costs. These days, alternative protein sources for aquafeeds include a variety of plant-based products including soybean and rapeseed meals as well as animal-based byproducts like bone and poultry meal. These options, however, come with a lot of drawbacks and put further burden on land-based agriculture systems that are already under pressure.

Aquaculture species' health, growth performance, and nutrient digestibility are all adversely impacted by plant-based products because of their high fiber content and antinutritional components (Okomoda et al., 2020). Furthermore, they are deficient in micronutrients and long-chain polyunsaturated fatty acids (PUFAs), which are critical for the growth and health of the majority of aquaculture products, particularly in the larval and nursery rearing stages (Malcorps et al., 2019). Finding sustainable, wholesome, and reasonably priced substitute components for aquafeed formulations is essential. A primary goal is to identify sources that, even at low inclusion rates, can mitigate the disadvantages of vegetable protein sources and substitute elements obtained from fish (Cottrell et al., 2020). When manufactured on a big scale, these elements must improve fish health, growth, and feed efficiency while being environmentally, socially, and economically viable.

3. Fishery and Aquaculture byproducts

According to Olsen et al. (2014), "fish wastes" encompass a range of undesirable fish species for human consumption, such as small-sized fish species, by-catch, and by-products of the aquaculture and fisheries sectors. Low-value products and processing waste that is not usually eaten (such as head, skin, fins, viscera, carapax, exoskeleton, shell, debris, etc.) are among them; these can make up more than 50% of the fish's body (Gasco et al., 2020). According to FAOSTAT (2014) and Caruso (2016), the aquaculture and fisheries sector generated over 20 million tons of garbage globally in 2014. Of this, the value of waste from marine fisheries alone was estimated to be around 25 million tons. An estimated 5.2 million tons are discarded annually in the European Union (EU) (Olsen et al., 2014; Lopes et al., 2015). As a result, the landing requirement law (Reg. (EU) No. 1380/2013; European Commission, 2013) was passed, with the intention of lowering the amount of unintended catches in the EU (Guillen et al., 2018).

Nonetheless, fish processing will rise along with efforts to enhance aquaculture's food production and fish fillet exports, producing a lot of solid waste from filleting and shellfish removal (Gamarro et al., 2013; Secci et al., 2016). Fish waste disposal is a big issue in many nations since improper disposal can have a negative effect on aquatic ecosystems (Arvanitoyannis & Kassaveti, 2008). This includes the discharge of organic wastes, which in aquatic environments can change the biodiversity and microbiota structure of benthic assemblages (Olsen et al., 2014). Thus, it is essential to handle fish waste and byproducts properly to reduce pollution issues caused by careless disposal. Including these undesirable products in animal and aquaculture species' feed is one method to manage them (EU, 2003; Ferraro et al., 2010). Fish and aquaculture byproducts fall under Category 3 byproducts that are permitted for inclusion in animal diets under EU Regulation 1069/2009, sustainably promoting public health and the environment (Gasco et al., 2020). A more sustainable method of fish farming could result from using discarded fishery by-products to create FMFO (Li et al., 2019). This would greatly lessen the strain on traditional fish stocks used to produce fishmeal (Garcia-Romero et al., 2014; Kim et al., 2014; Gisbert et al., 2018).

Through the extraction of high-value nutritional matter from waste materials, this environmentally friendly method lowers feed formulation, production, and administration costs, resulting in financial benefits (Li et al., 2019). Another method for turning waste into fish protein hydrolysates, which are short-chain peptides and amino acids, is to process fisheries waste by enzymatic hydrolysis (Gasco et al., 2020). Because fisheries and aquaculture by-products (F&A by-products) are rich in macro- and micronutrients, including them in fish meals has benefits (Olsen et al., 2014; Elhag et al., 2022). F&A by-product feeding has been shown to have positive biological effects on fish, as evidenced by studies that show enhanced growth, immune system function, and antioxidant activity (Kotzamanis et al., 2007; Ambigaipalan & Shahidi, 2017).

3.1 Food Wastes

Another unconventional source of protein that can be utilized to make aquafeed is food waste (Bake et al., 2009; Nasser et al., 2018). Food materials that are thrown away, both cooked and raw, as well as food leftovers that are recycled (USEPA, 2012; Cheng et al., 2015; Choi et al., 2016). According to estimates from the FAO (2015), almost one-third of the world's food production—1.3 billion tons—is wasted when it is produced for human consumption. Meanwhile, famine affects an estimated 925 million individuals in emerging and low-income nations (Buckle, 2015). Foods that are not consumed usually wind up in landfills, where they break down and release methane gas and landfill leachates (Ishigaki et al., 2002).

Twenty times as likely to cause global warming as carbon dioxide is methane, a greenhouse gas. Another way to deal with food waste is to incinerate it, however this process is ineffective because of the trash's high moisture content and can pollute the environment (Xiao et al., 2007; Zhuang et al., 2008). Additionally, food wastes are recycled to create fertilizer and energy. This approach is limited, though, by the limited capacity of recycling businesses and the availability of more reliable, cleaner, and longer-lasting alternative raw materials (HKEPD, 2012). Thus, creative ways to make use of these underutilized, low-cost sources of high-nutrient protein are required.

3.2 Insects

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As a result, there has been significant investment in insect-rearing start-up businesses globally; by early 2019, over 42 European businesses had been founded and were actively raising a variety of insects (Mancuso et al., 2019). Around 50,000 tons of insects were produced annually worldwide at the time, and expansion was anticipated (IPIFF, 2019; Mancuso et al., 2019).

At least sixteen insect species—of the over one million known species worldwide—have been studied as potential substitutes for fish used in aquaculture (Henry et al., 2015; Nogales-Mérida et al., 2019; Guerreiro et al., 2020). Alfiko et al. (2022) state that just eight of these species have demonstrated encouraging outcomes in research. A few of these are the housefly maggot and pupae Musca domestica (Emeka & Oscar, 2016; Kolawole & Ugwumba, 2018; Achionye-Nzeh & Ngwudo, 2021), black soldier fly Hermetia illucens (Ji et al., 2015; Nuswantoro & Rahardjo, 2018; Wu et al., 2021), and mealworms (including yellow mealworms Tenebrio molitor and lesser mealworm Alphitobius diaperinus).

In addition to having equal nutritional value, insect-based feed resources are being investigated as possible substitutes for pricey conventional ingredients due to a number of additional benefits (Barroso et al., 2014; Henry et al., 2015; Nogales-Mérida et al., 2019). Because insects feed on wastes and byproducts with a high conversion efficiency, these benefits include a decreased production and processing impact on the environment as well as the possibility of turning waste into money (Zarantoniello et al., 2018).

3.3 Single cell organisms

Because of their nutritional makeup and efficacy as feed, single-cell organisms (SCO), such as bacteria, fungus, microalgae, and combinations like biofloc, have shown to be a great alternative to fishmeal and fish oil (FMFO). Fish growth performance, immunity, health, and quality have all improved as a result of the addition of SCO to feed (Shah et al., 2018; Richard et al., 2021). Because SCOs grow quickly, consume little fresh water, and do not require agricultural area for growth, their production is seen as sustainable. They can also be made from aquaculture wastes and non-food waste streams (Viegas et al., 2021; Albrektsen et al., 2022).

One benefit of using organic substrates such as methane, methanol, syngas, carbon dioxide, hydrogen, and second-generation sugars is that bacteria may grow quickly on them (Matassa et al., 2020). Gas-based fermentation technique generates methanotrophic bacterial meal by using natural gas as a source of energy and carbon. Natural gas is a cheap and plentiful resource, making protein manufacturing from it a feasible large-scale alternative. Similar to fishmeal, bacterial meal has up to 80% crude protein (mean = 60%) and roughly 10% fat (Glencross et al., 2020; Albrektsen et al., 2022). According to studies, diets containing up to 55% methanotrophic bacterial meal for salmon and 38% for trout either improved feed efficiency and growth performance or had no negative impact on growth. (Aas et al., 2006; Øverland et al., 2010; Hardy et al., 2018). However, long-term feeding with high concentrations of bacterial meal has been associated with decreased protein digestibility and reduced growth and survival in salmon (Storebakken et al., 2004).

A number of commercial methanotroph-based bacterial meals should soon be accessible thanks to recent developments in gas-based fermentation technology (Albrektsen et al., 2022). According to Tlusty et al. (2017) and Hamidoghli et al. (2019), shrimp have also demonstrated favorable reactions to a variety of bacterial meals, with inclusion rates ranging from 10% to total replacement of fishmeal in diets. Shrimp's growth performance, feed conversion ratio, and resistance to disease and stress have all improved with the addition of a new microbial protein source: photoheterotrophically developed purple non-sulfur bacteria (such Rhodopseudomonas palustris and Rhodobacter capsulatus) (Alloul et al., 2021). Furthermore, according to Delamare Deboutteville et al. (2019), these purple phototrophic bacteria that were created from wastewater replaced up to 66% of fishmeal in sea bass diets without having a negative impact on fish performance.

3.4 Seaweed

Global production of seaweed (macroalgae) aquaculture reached 32 million tons in 2018, accounting for 51% of global mariculture production and having a market value of more than USD 11 billion (FAO, 2020). At the moment, Asia accounts for more than 99% of seaweed agriculture, with Africa contributing a growing percentage (FAO, 2020). Human consumption accounts for over 95% of the seaweed produced, including Japanese wakame and kelp (Ferdouse et al., 2018). Seaweed has recently come to light for its potential for bioremediation, offering an extremely sustainable manufacturing situation. Seaweed reduces environmental effect by converting nutrient-rich wastewater sources from agriculture, aquaculture, and power generation into biomass through the integration of seaweed cultivation and these sources (Ge et al., 2017; Neveux et al., 2018; Arumugam et al., 2019).

The taxonomic category (red, green, or brown) and seasonality have a substantial impact on the nutritional content of seaweed (Wan et al., 2019). The ranges for protein concentration in red seaweeds are 6–38%, in green seaweeds, 3–35%, and in brown seaweeds, 2–17%, and in lipid ranges, <1–13%, <1–3%, and <1–10%, respectively. The biomass protein content of green, brown, and red seaweeds has been successfully doubled recently through enrichment processes, which have also produced useful by-products like carrageenan, salt, ulvan, fucoidan, laminarin, phenolics, and carotenoids (Holdt & Kraan, 2011; Magnusson et al., 2019; Øverland et al., 2019; Gordalina et al., 2021; Aasen et al., 2022). Furthermore, it has been demonstrated that fermentation is a viable bio-refinement method for seaweed, increasing protein by a large amount, tripling in vitro digestibility, and producing advantageous by-products including organic acids, antioxidants, phenolics, and flavonoids (Fleurence et al., 2018; Ang et al., 2021).

3.5 Low-trophic Marine Animals

Particularly interesting marine creatures to consider as FMFO substitutes are polychaetes, amphipods, and mussels. These low-trophic organisms feed on primary producers found in the marine environment, such as bacteria, algae, phytoplankton, and organic waste. Filter-feeding mollusks, mussels (Perna viridis) and Mytilus edulis (blue) comprise around 56% of marine animal aquaculture production at present (FAO, 2020). Because they can thrive in nutrient-rich habitats and turn waste nutrients into protein without the need for additional feed, mussels are excellent as bioremediators. Mussels are grown in maritime waters; they do not use freshwater or terrestrial resources. Their essential amino and fatty acid profiles are similar to fishmeal, and they have a high protein content (50–70% DW) and high lipid content (5–16% DW) (Jusadi et al., 2021). In the diets of turbot and catfish, mussel meal has demonstrated encouraging effects when used in part instead of fishmeal (Weiss, 2017; Wang et al., 2020). The production cost of high-quality mussel meal was projected to be USD 1.60 kg-1 in a recent price analysis of larger, faster-growing tropical mussels like Perna perna. This is comparable

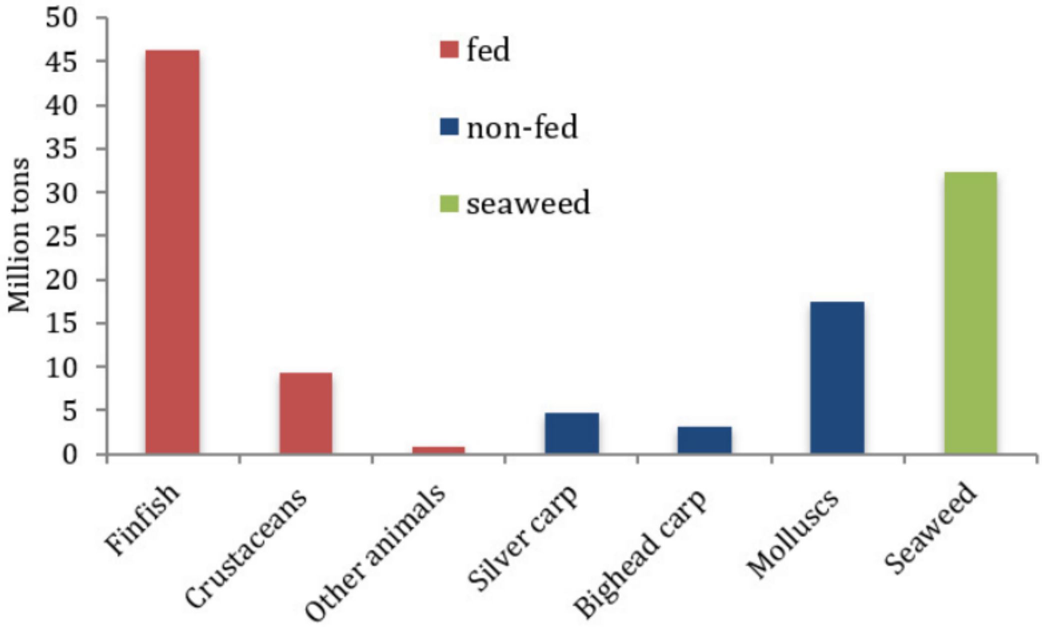

Figure 1: Total aquaculture production values in 2018 of fed and non-fed fish, including seaweed. Adapted from data released in the FAO (2020) report

to the current international price of USD 1.50 kg-1 for fishmeal (Suplicy, 2020). ***Source: Shahin et al., 2023***

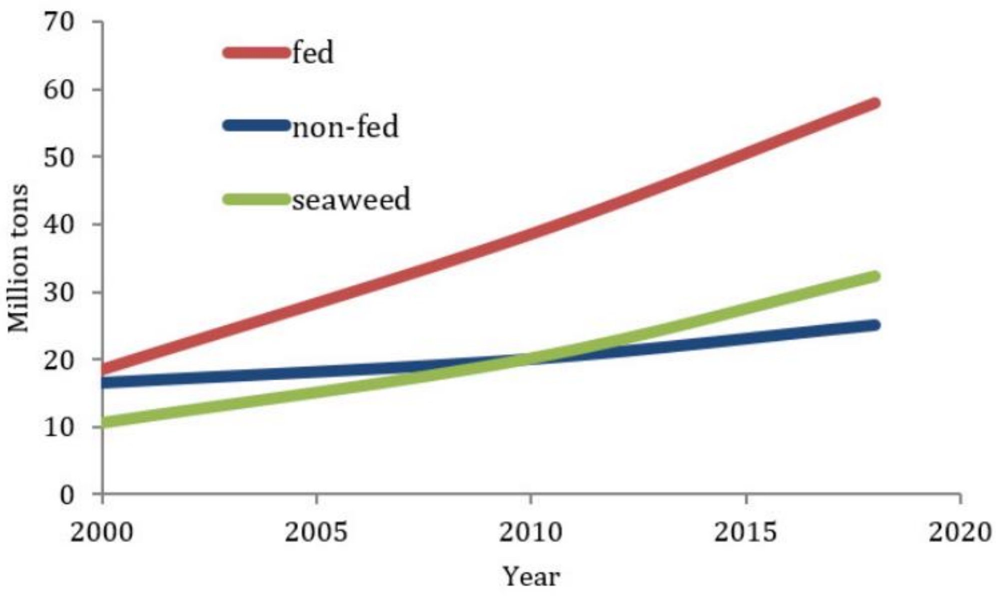

Figure 2: Historic growth of fed and non-fed total aquaculture production values, including seaweed. Adapted from data released in the FAO (2020) report

Source: Shahin et al.,2023

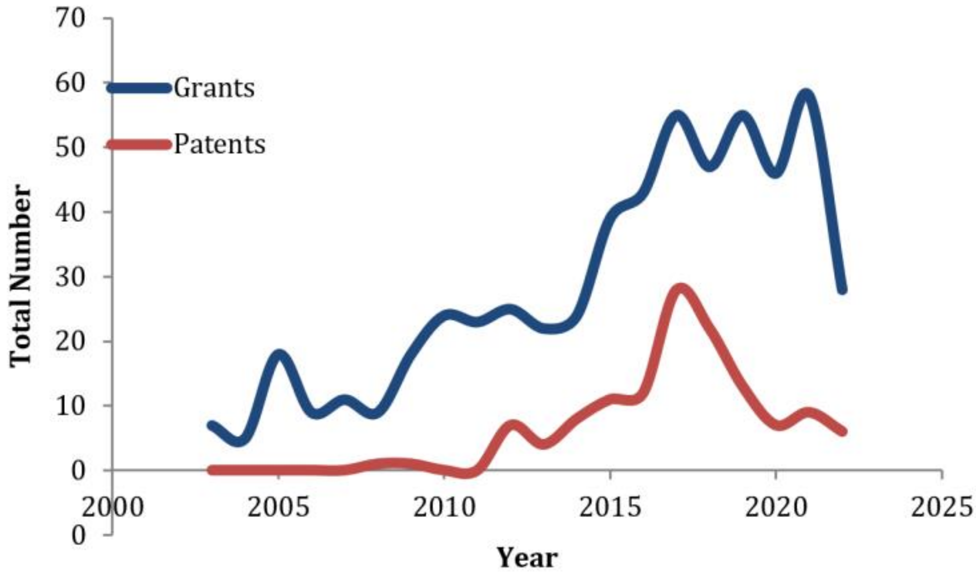

Figure 5: Research grants awarded and patents licensed with respect to sustainable aquaculture feed over the last 20 years. Adapted from data retrieved from Digital Science. (2018-) Dimensions [Software] available from https://app.dimensions.ai. Accessed on (May 16, 2023), under license agreement

Source : Shahin et al.,2023

Reference:

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Chapter: 10

Government Initiatives and Policies Supporting Climate-Resilient Aquaculture Systems through the Development of Sustainable Fish Feeds

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Abstract

Aquaculture plays a vital role in achieving Sustainable Development Goals (SDGs) by promoting health, livelihoods, and social empowerment. The Indian government multi-faceted approach to promoting climate-resilient aquaculture systems includes research funding, financial incentives, capacity building, and public-private partnerships. These efforts focus on developing sustainable fish feeds using alternative ingredients such as plant proteins, insect meals, and algae, reducing reliance on fishmeal and fish oil. Training programs, public awareness campaigns, and regulatory standards support the adoption of innovative feed technologies. Globally, similar initiatives are supported by the EU, the U.S., Canada, China, Australia, Japan, and international organizations like the FAO. These initiatives aim to reduce environmental impact due to aquaculture practices, ensure long-term viability, and promote sustainable and cost-effective practices. Collaborative efforts between governments, research institutions, and private sectors drive innovation in sustainable feed development, fostering a more resilient and environmentally responsible aquaculture industry.

1. Introduction

Aquaculture plays a crucial role in promoting health, livelihoods, and social empowerment, and significantly contributes to several Sustainable Development Goals (SDGs), including the reduction of hunger and poverty. The development of climate-resilient aquaculture systems is essential to ensure the sustainability, productivity, and adaptability of aquaculture practices in the face of climate change. These systems are designed and managed to integrate various strategies and technologies, making aquaculture more robust against environmental stressors. A key element in sustainable aquaculture is the development and discovery of novel fish feed ingredients. Since feed costs account for more than half of aquaculture production expenses, various feed reduction schemes have been implemented. One promising approach is the adoption of a circular bioeconomy in aquafeed use and development. This involves the use, reuse, and recycling of non-conventional, innovative fish feed ingredients, such as by-products, agricultural industry processing wastes, and natural raw materials that do not compete with human food consumption. These revalorized feed ingredients are often cost-effective, environmentally sustainable, and pose no significant negative effects on the environment. Before novel feed ingredients are utilized, they undergo rigorous testing and evaluation. Two primary methods are used to assess the feasibility and potential of candidate feed ingredients: systematic review and meta-analysis, and in vivo testing. Each method has its own advantages and limitations.

2. Growing number of Sustainable Feed Ingredients

The growth of aquaculture has historically led to a dependence on limited feed ingredients, particularly fishmeal and fish oil. However, alternatives are being explored and adopted as they become technically and economically feasible. In 2016, marine proteins accounted for only 14.5% of feed, while plant protein increased to 40.3%. Carbohydrates, used as binding agents, have consistently made up about 10.3% of the feed, and micro-ingredients such as vitamins, minerals, phosphorus sources, astaxanthin, and crystalline amino acids are slowly increasing. The composition of fish feed has evolved from being dominated by fishmeal (65%) in 1990 to primarily consisting of vegetable raw materials (73%) in 2020. Despite the importance of fishmeal and fish oil, concerns about their sustainability and rising global prices are driving the search for alternative feed sources. The finite supply of fishmeal and fish oil may constrain aquaculture development, as the stagnation of wild fisheries and the growth of the aquaculture sector could lead to increased harvesting of wild stocks and unsustainable fishing practices. The sustainability of the aquaculture sector is also linked to the sustainable supply of terrestrial resources. Herbivorous and omnivorous species dominate aquaculture, relying on terrestrial sources such as soybeans, maize, rice, and wheat, which are also vital for terrestrial livestock and human consumption.

Traditionally, diets for carnivorous fish contained over 50% fishmeal and oil. However, these percentages have decreased as alternative feed sources have been developed. Research has shown that other combinations of ingredients can provide the essential nutrients required by fish, allowing a reduction in fishmeal and fish oil use. These alternative feeds not only offer environmental benefits but also present economic advantages for farmers due to their lower costs.

Sustainable replacement options for fishmeal and fish oil include meals and oils from plants, fish processing trimmings, yeast, insects, and seaweed. Already in use are proteins from soybeans, corn, peas, and wheat, and oils from soybean, canola, and flaxseed. These replacements have significantly reduced reliance on raw marine materials for aquaculture feeds. For instance, a Nature article reported that the use of fish-based ingredients in Atlantic salmon feed declined from 90% in the 1990s to 25% in 2020. Two critical components of sustainable fish feed are feed efficiency and feed ingredients. High-quality feed and effective fisheries management can improve feed efficiency, resulting in better fish growth with less feed consumed. Feed ingredients must be evaluated for their impact on fish health and the environment, seeking alternatives that are more effective and environmentally friendly.

Perfect substitutes for fishmeal and fish oil have not yet been identified, as no alternative source fully meets both nutritional and economic requirements. Fishmeal remains a crucial ingredient for marine and carnivorous species, while fish oil is irreplaceable in the feed for all types of fish. Formulated pelleted feed, consisting of a variety of pressed ingredients, has become the primary source of nutrition for farmed fish. Although no single alternative can completely substitute fishmeal or fish oil, the use of substitute ingredients in pellets is likely to increase in the future.

3. Government Initiatives and Policies Supporting Climate-Resilient Aquaculture Systems

The governments policies and initiatives play a crucial role in transitioning the aquaculture industry towards more sustainable and climate-resilient practices. These efforts help to mitigate the environmental impact of aquaculture and ensure the long-term viability in the face of climate change. Governments worldwide are increasingly recognizing the need to support climate-resilient aquaculture systems. Developing sustainable fish feeds is a crucial part of these initiatives, as feed is a major component of aquaculture's environmental footprint.

The Government of India in collaboration with various states has focused on the issues affecting aquaculture and launched a national action plan "Mission Blue Revolution: Integrated National Fisheries Action Plan 2016" covering state-specific needs and addressing food security, livelihoods and rural development issues. Although, India has been performing well in the aquaculture sector, it has still not achieved its potential. A main area of focus is increasing the availability of good quality fingerlings and Mission Fingerling is now operational to achieve this. Another focus is conserving good quality germplasm, and a National Brood Bank has been established to provide improved varieties of breeder seeds. A third focus is to protect the natural fish wealth and in line with this public awareness programmes and rehabilitation of natural broodstock are being carried out through river ranching in special areas of the Ganges and its tributaries under the Namami Ganges National Action Plan to clean the river. Recirculating aquaculture systems (RAS) are being established for intensification of aquaculture to mitigate the impact of climate changes. Moreover, the farming of climate-resilient food fishes in captivity is also being promoted. Promotion of cage/pens in wetlands is also gaining momentum. Culture of endemic varieties of hardy fishes such as Indian catfishes and murrels is also a feature of government efforts. Ensuring the flow of funds and co-ordination through convergence with other National Rural Development Schemes such as the National Agriculture Development Programme, also known as Rashtriya Krishi Vikas Yojana (RKVY), which are also promoting inland aquaculture and the inland fisheries sector.

The key government initiatives and policies that support the development of sustainable fish feeds to promote climate-resilient aquaculture systems:

3.1 Grants and Subsidies: Governments provide grants and subsidies to encourage research into sustainable fish feed alternatives. For instance, the U.S. Department of Agriculture (USDA) and the National Oceanic and Atmospheric Administration (NOAA) offer funding for innovative aquaculture research projects, including those focused on sustainable feeds. The Government of India provides various grants and subsidies to support the development of sustainable fish feeds, aiming to enhance the aquaculture sector productivity and environmental sustainability. PMMSY offers subsidies for the establishment of fish feed mills, especially those focusing on sustainable and innovative feed production. PMMSY also offers Grants for research projects aimed at developing alternative feed ingredients, such as plant-based proteins, insect meals, and algae-based feeds. Under Blue Revolution Scheme, grants are provided for demonstration projects showcasing the benefits of sustainable fish feeds and promoting their adoption among fish farmers. National Fisheries Development Board (NFDB) runs specific programs to support the development and distribution of high-quality, sustainable fish feeds. This includes funding for pilot projects, research, and capacity-building activities. Financial assistance is available for the procurement and utilization of innovative and sustainable feed ingredients. Central Sector Scheme on Blue Revolution offers Grants for the development of infrastructure necessary for sustainable feed production, including feed mills and storage facilities. These grants and subsidies aim to promote the development and adoption of sustainable fish feeds, ultimately contributing to the growth of a more resilient and environmentally responsible aquaculture sector in India.

3.2 Partnerships with Research Institutions: The Government of India actively collaborates with various research institutions to develop sustainable fish feeds, leveraging scientific expertise and technological advancements. These partnerships focus on creating alternative protein sources, enhancing feed efficiency, and minimizing the environmental impact of aquaculture. Key initiatives include ICAR work through CIFE, CIBA, CIFA, CIFRI and CMFRI which target different aquaculture environments, and NFDB funding of research projects and training programs.

3.3 Regulations and Standards: Many countries have established regulations that govern the types of ingredients that can be used in fish feeds. European Union has stringent regulations on the use of fishmeal and fish oil, encouraging the use of alternative proteins like insect meal and plant-based proteins. Programs like the Aquaculture Stewardship Council (ASC) and the Global Aquaculture Alliance Best Aquaculture Practices (BAP) certification include criteria for sustainable feed sourcing. The Government of India has established various regulations and standards to promote sustainable fish feed development, ensuring aquaculture practices are environmentally responsible, economically viable, and socially beneficial. Key measures include quality and safety standards by the Food Safety and Standards Authority of India (FSSAI), aquafeed specifications and quality control by the Bureau of Indian Standards (BIS), environmental regulations and feed management practices by the Coastal Aquaculture Authority (CAA), national policies and subsidies by the Department of Fisheries, research and best practices by the Indian Council of Agricultural Research (ICAR), regulatory frameworks by the National Aquaculture Development Authority (NADA), and environmental protection laws mandating waste management and sustainable resource utilization. These regulations and standards by the Government of India aim to ensure the development of sustainable fish feeds, promoting environmental stewardship, economic viability, and social responsibility in the aquaculture sector.

3.4 Training Programs, Public Awareness Campaigns and Outreach Activity: Training programs, public awareness campaigns, and outreach activities are integral to promoting the development of sustainable fish feeds in India. These initiatives aim to educate stakeholders, enhance skills, and foster community engagement in sustainable aquaculture practices. These initiatives, led by institutions like ICAR and state agricultural universities, provide technical workshops and capacity-building sessions to promote innovative feed formulations and eco-friendly aquaculture practices such as recirculating aquaculture systems (RAS). Public awareness efforts by agencies like FSSAI and CAA emphasize health benefits, environmental impact mitigation, and regulatory compliance related to sustainable feed options. Outreach activities, including farmers field days and extension services facilitated by NGOs and agricultural officers, demonstrate sustainable feed technologies and encourage their adoption. These comprehensive efforts aim to enhance knowledge, improve feed management practices, and foster community engagement to ensure the widespread implementation of sustainable fish feeds across India aquaculture sector.

3.5 Supporting Alternative Feed Development: Government initiatives in India promote the development of alternative feeds for aquaculture to enhance sustainability and reduce reliance on fishmeal and fish oil. Programs like Pradhan Mantri Matsya Sampada Yojana (PMMSY) provide subsidies and incentives for research and infrastructure development. The National Fisheries Development Board (NFDB) funds projects and offers technical support for adopting alternative feed technologies. Additionally, the Rashtriya Krishi Vikas Yojana (RKVY) supports schemes aimed at improving feed production and sustainability practices. Regulatory bodies like the Coastal Aquaculture Authority (CAA) set guidelines for sustainable feed ingredients and best management practices. Research institutions and capacity-building programs further drive innovation and awareness, fostering a shift towards environmentally friendly and economically viable aquaculture practices in India.

3.6 Insect Farming Initiatives: Some governments have invested in the development of insect farming as a sustainable protein source for fish feeds.

- **European Union:** The EU has funded various projects under the Horizon 2020 research program to develop insect-based feed ingredients, aiming to create sustainable and eco-friendly alternatives to traditional fishmeal and fish oil.
- **Canada:** Through its AgriInnovate Program, the Canadian government supports innovative agricultural projects, including insect farming for animal and aquaculture feeds, focusing on improving sustainability and reducing environmental impacts.
- **China:** The Chinese government has invested in large-scale insect farming initiatives to supply protein for aquaculture, leveraging its vast agricultural sector to scale production and meet growing demand.

3.7 Algae and Plant-Based Feed Research: Government initiatives globally support the development of algae and plant-based feed ingredients to reduce reliance on wild-caught fishmeal and fish oil:

- **United States:** The U.S. Department of Agriculture (USDA) provides grants for research into algae-based feed ingredients, aiming to enhance the sustainability of aquaculture practices. Collaborations with universities and private sector partners are key components of these efforts.
- **Australia:** The Australian government, through its Fisheries Research and Development Corporation (FRDC), funds research on plant-based and algae-based feed alternatives, promoting sustainable aquaculture and reducing the industry's environmental footprint.
- **Japan:** Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) supports research on alternative feed ingredients, including algae and plant-based proteins, to ensure sustainable and efficient aquaculture practices.

4. Public-Private Partnerships Supporting Sustainable Feed Development

Public-private partnerships (PPPs) play a crucial role in advancing the development of sustainable feeds for aquaculture. These collaborations leverage the strengths of both sectors to drive innovation, share knowledge, and implement scalable solutions. Governments and organizations worldwide are leveraging public-private partnerships to promote sustainable aquaculture feeds. The EU collaborates with the European Former Foodstuff Processors Association (EFFPA) to use former foodstuffs in animal feeds, reducing waste. In the U.S., the USDA partners with companies like Calysta and Cargill to develop alternative protein sources, such as microbial proteins and insect meals. Canada works with Enterra Feed Corporation on insect-based feeds. In India, National Fisheries Development Board (NFDB) collaborates with private companies to promote cost-effective, sustainable feed technologies. China partners with tech firms to scale up insect farming and algae-based feed production. In Australia Fisheries Research and Development Corporation (FRDC) works with universities and enterprises on plant-based and algae-based alternatives. Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) collaborates with industry leaders on algae and plant protein solutions. The FAO partners with global companies to adopt innovative feed ingredients like insects and algae. These public-private partnerships are instrumental in advancing sustainable feed development, combining resources and expertise to create innovative and eco-friendly solutions for the aquaculture industry.

3. International Collaboration

Governments and international organizations worldwide are implementing initiatives and policies to support the development of sustainable and alternative feeds for aquaculture. These efforts aim to enhance sustainability, reduce environmental impacts, and ensure the long-term viability of the aquaculture industry. The European Union funds research on insect and algae-based feeds via Horizon 2020 and Horizon Europe Programs, while the Common Fisheries Policy supports sustainable practices. In the U.S., the USDA and NOAA provide grants and support for eco-friendly feed research. Canada AgriInnovate Program and Sustainable Fisheries Framework focus on innovative and sustainable feed projects. China National Agricultural Science and Technology Innovation Program invests in large-scale sustainable feed initiatives, supported by its

Five-Year Plans. Australia Fisheries Research and Development Corporation and National Aquaculture Strategy promote plant-based and algae-based feed alternatives. Japan Ministry of Agriculture, Forestry and Fisheries, alongside the Blue Growth Initiative, emphasizes sustainable feed ingredient research. The FAO Blue Growth Initiative and Technical Cooperation Programs globally encourage sustainable aquaculture practices. These combined efforts aim to reduce environmental impacts and ensure the long-term viability of the aquaculture industry. These international initiatives and policies collectively aim to reduce the environmental footprint of aquaculture, enhance feed efficiency, and promote the use of sustainable and innovative feed ingredients across the globe.

6. Conclusion

The Indian government adopts a comprehensive approach to promote climate-resilient aquaculture systems through sustainable fish feed development. This involves a combination of research funding, financial incentives, capacity building, and public-private partnerships. Key initiatives and policies aim to enhance the sustainability, productivity, and resilience of the aquaculture sector in response to climate change. These efforts include policy support, financial incentives, and extensive research and development, designed to reduce the environmental impact of aquaculture while ensuring its long-term viability. Overall, these multifaceted initiatives aim to bolster the sector resilience, productivity, and sustainability amidst climate challenges.

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Chapter: 11

Fish Feed Formulation Research Using Zebrafish Models: Enhancing Sustainable and Climate Resilient Aquaculture

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1. INTRODUCTION

Food is the life support system for all living beings, providing essential nutrients and energy required for survival. It fuels metabolic processes, sustains bodily functions, and supports growth and repair. A balanced diet, rich in fruits, vegetables, whole grains, lean proteins, and healthy fats, is crucial for optimal physical and mental well-being. Among various food sources, fish stands out due to its unique combination of high-quality protein, healthy fats, and vital nutrients. Fish is particularly notable for being low in fat and rich in omega-3 fatty acids, vitamins D and B2, calcium, phosphorus and essential minerals like iron, zinc, iodine, magnesium, and potassium.

Consuming fish regularly offers numerous health benefits, including reduced risk of cardiovascular diseases, improved cognitive function, and support for healthy aging. Consequently, the global demand for fish continues to rise. To meet this growing demand and alleviate pressure on wild fish stocks, aquaculture or fish farming has emerged as a sustainable solution. Aquaculture not only addresses the depletion of wild fish due to overfishing and environmental degradation but also provides a reliable source of healthy protein, particularly in developing countries.

A critical component of aquaculture is the formulation of nutritious fish feed, designed to meet the specific nutritional needs of farmed fish for optimal growth and health. This feed typically includes a balanced mix of proteins, lipids, carbohydrates, vitamins, and minerals, often derived from fishmeal, plant proteins, and oils rich in omega-3 fatty acids. The inclusion of probiotics, vitamins, and immune enhancers further promotes fish health and disease resistance.

Ensuring the quality and safety of fish feed is paramount, as it directly influences the nutritional value and safety of farmed fish for human consumption. Contaminated fish feed can introduce harmful substances such as heavy metals, pesticides, mycotoxins, and industrial pollutants (e.g., PCBs, PAHs) into the food chain. When humans consume fish exposed to these contaminants, they face significant health risks including neurological damage, genetic mutations, developmental issues, cancer, and endocrine disruption. Chronic exposure to these toxins can also impair immune function and increase susceptibility to diseases. To protect human health, stringent regulation, regular monitoring of fish feed, and sustainable agricultural and industrial practices are essential to prevent contamination and ensure the safety of farmed

2. CASE STUDIES ON FEED ADULTERATION

2.1 Melamine Contamination in Fish Feeds

In the United States, melamine was found in wheat gluten and rice protein concentrate imported from China, used in manufacturing fish feeds. High levels of melamine caused renal failure and death in fish due to the formation of insoluble crystals in their kidneys. The contaminated fish posed a risk to human health if consumed, leading to a significant public health scare. The scandal prompted stricter regulations on feed ingredients and mandatory testing for melamine in animal feeds.

2.2 Antibiotic Residues in Fish Feeds

In Southeast Asia, a survey revealed that several fish feed samples contained unauthorized antibiotics, including chloramphenicol and nitrofurans, which are banned in many countries. Continuous exposure to antibiotics can disrupt the gut microbiota and weaken fish immune systems. Consumers risked exposure to antibiotic residues, which could contribute to antibiotic resistance. The discovery led to increased monitoring and enforcement of antibiotic use in aquaculture, including the implementation of stricter residue limits.

2.3 Substitution of High-Quality Ingredients with Low-Quality Alternatives

In Europe, an investigation into fish feeds revealed that some manufacturers were substituting fish meal and fish oil with soybean meal and palm oil without proper labelling. The altered nutritional profile led to poor growth rates and increased disease susceptibility in farmed fish. Mislabelling damaged trust between producers and consumers, prompting authorities to introduce stricter labelling regulations and mandate transparent ingredient disclosure.

2.4 Heavy Metal Contamination

In Asia and Africa, several aquaculture farms reported high mortality rates among fish, which were later traced back to heavy metal contamination in the feed. Heavy metals caused severe organ damage and mortality in fish. Consumption of contaminated fish posed serious health risks to consumers. The incident led to the establishment of maximum permissible levels of heavy metals in fish feeds and more stringent sourcing of raw materials.

2.5 Inclusion of Harmful Additives and Fillers

In Africa, fish farmers reported stunted growth and poor feed conversion ratios in their stocks. Investigation revealed that the feeds contained high levels of indigestible fillers like sawdust and sand. The fillers provided no nutritional value and caused digestive issues in fish. Farmers faced financial losses due to poor growth performance and increased feed costs. Governments implemented stricter feed quality standards and regular inspections to prevent such practices.

3. TOXICITY SCREENING OF FISH FEEDS

Ensuring the quality and safety of fish feed is crucial to prevent adverse genetic effects and maintain the health and sustainability of fish populations in aquaculture. Therefore, toxicity screening of fish feeds before cultured fish consumption is essential to ensure food safety and also to protect human health. Screening involves testing for contaminants such as heavy metals, pesticides, toxins, and pathogens that may be present in feed ingredients. These screenings assess the potential risks associated with feed consumption and help prevent the accumulation of harmful substances in fish tissue. By identifying and mitigating potential hazards in fish feeds, toxicity screening safeguards both the health of cultured fish and the consumers who rely on them for sustenance.

4. ZEBRAFISH AS A MODEL ORGANISM

Zebrafish serve as a valuable model organism for toxicity screening of fish feeds due to their small size, rapid reproduction, and transparent embryos, allowing for easy observation of developmental processes. Their genetic similarity to humans and sensitivity to environmental toxins make them ideal for assessing the safety of fish feeds. Zebrafish embryos can quickly reveal developmental abnormalities or toxic effects caused by feed ingredients, providing insights into potential risks to fish health and human consumption. By using zebrafish in toxicity screening, researchers can efficiently evaluate the safety of fish feeds, ensuring the production of safe and nutritious cultured fish for human consumption.

5. APPLICATIONS OF ZEBRAFISH IN FISH FEED FORMULATION RESEARCH

Zebrafish (*Danio rerio*) have become a valuable tool in fish feed formulation research due to their unique biological characteristics and amenability to laboratory experimentation. Zebrafish can be effectively utilized as an animal model in feed formulation research for the following reasons:

1. **Genetic Similarity and Translational Value**: Zebrafish share significant genetic similarities with humans, making them an excellent model for studying the effects of different feed formulations on gene expression and health outcomes.

2. **Nutritional Requirement Studies**: Zebrafish can be used to determine the nutritional requirements of fish, ensuring that feed formulations meet the dietary needs for optimal growth and health.

3. **Digestibility and Absorption Studies**: Researchers can study the digestibility and absorption of various feed ingredients in zebrafish to develop more efficient and nutritious fish feeds.

4. **Evaluation of Alternative Ingredients**: Zebrafish can be used to test the efficacy and safety of alternative ingredients in fish feeds, promoting the use of sustainable and cost-effective feed components.

5. **Functional Additives and Supplements**: The effects of functional additives and supplements, such as probiotics and immune enhancers, can be evaluated using zebrafish to improve fish health and resistance to diseases.

6. **Toxicology and Safety Assessment**: Zebrafish are ideal for toxicity screening of feed ingredients, identifying potential contaminants, and ensuring the safety of fish feeds for human consumption.

7. **Behavioural Studies**: Zebrafish behaviour can be monitored to assess the impact of different feed formulations on fish welfare and overall well-being.

6. MOLECULAR AND GENETIC STUDIES USING ZEBRAFISH

Zebrafish can be used as a tool in molecular and genetic studies in fish feed formulation research in several ways:

1. **Gene Expression Analysis**: Zebrafish can be used to study changes in gene expression in response to different feed formulations, providing insights into the molecular mechanisms underlying nutritional and toxicological effects.
2. **Functional Genomics**: Researchers can utilize zebrafish for functional genomics studies to identify key genes involved in nutrition and toxicity responses.
3. **Epigenetic Studies**: Zebrafish provide a model for studying epigenetic modifications induced by different dietary components and contaminants in fish feeds.
4. **Genetic Mapping and QTL Analysis**: Quantitative trait loci (QTL) analysis in zebrafish can help identify genetic factors influencing growth, health, and resistance to diseases.
5. **Comparative Genomics**: Zebrafish can be used for comparative genomics studies to understand evolutionary conservation and divergence of nutritional and toxicological pathways.
6. **CRISPR/Cas9 Genome Editing**: Advanced genome editing techniques, such as CRISPR/Cas9, can be applied in zebrafish to study the functional roles of specific genes in nutrition and toxicity responses.

7. CONCLUSION

Zebrafish serve as a versatile and invaluable tool in fish feed formulation research, offering insights into nutritional requirements, digestibility, alternative ingredients, functional additives, safety and feeding behaviour. Leveraging the genetic, physiological and behavioural characteristics of zebrafish enhances our understanding of fish nutrition and contributes to the development of sustainable aqua feeds. By harnessing the translational value of zebrafish research, we can advance aquaculture practices, promote fish health and welfare, and ensure the long-term sustainability of the aquaculture industry. Ensuring the quality and safety of fish feed through rigorous toxicity screening using zebrafish is essential for providing safe and nutritious cultured fish for human consumption, ultimately contributing to human health and well-being.