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Review Article

Natural Nutritional Ingredients hold the key to a Sustainable Future in Aquaculture: Awakening the Indian Sundarbans for self preservation

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ABSTRACT

With the ever expanding aquaculture sector, the demand for feed rises. This has lead to the sprouting of a multitude of manufacturers, some even introducing spurious additives to make up for lack of protein content in feed and hence bridging a gap between feed formulators and aqua-farmers. The over exploitation of the aquaculture trade has adverse impacts on feed availability, disease and the ecology. The use of antibiotics have created further imbalance with drug resistant microorganisms. In an ongoing effort to reduce the use of crude feed and control of disease and drugs, FAO has advised the development of certain nutritional supplementations for a sustainable and profitable aquaculture process. As a response to this we have identified key natural nutritional components that may provide sustenance in aquaculture. Amino acids, allicin (from garlic) and sea weed extract have shown to provide a full spectrum of growth, immunity, antioxidant values and pose as alternatives to pharmaceutical antibiotics. We look into the prospects of these supplements as a sustainable solution in aquaculture nutrition by reviewing the work done so far by investigators. The coastal aquaculture communities of the Indian Sundarbans need to adopt such options. The challenge is to build trust with aquafarmers for them to accept sustainability programmes with sound management action to envision a safer aquaculture production.

Key words: Sustainable aquaculture, amino acid, allicin, sea weed, Indian Sundarbans

INTRODUCTION

FAO predicts the global aquaculture production will exceed 100 million tonnes by 2020. With a current world production at 66 million tonnes ¹, India contributes over 4.2 million tonnes ranking them the world's second largest aquaculture producer. China sits at the top of the table at 41 million tonnes. With a continuous rising demand in fish and other aquaculture produce a reduction in operation costs and sustainability is of utmost importance.

With fast disappearing fresh water areas, aquaculture development gradually found its way into marine and brackish water systems ². Aquaculture of shrimp and

piscivorous fishes has several drawbacks. Intensive monoculture causes disaster to coastal ecosystems by way of deteriorating the water quality, mangrove deforestation etc ^{3, 4, 5}. As a result of over exploitation, GreenPeace ⁶ has placed tiger shrimp (*Peneaus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*) in the seafood red list category for unsustainable fishing practices involved. This means the western food fish market is limiting their purchase of tropical prawns, hence adversely affecting our coastal aquaculture commerce. Intensive stocking and excessive feed results in stress and disease related loss. White spot syndrome is a relevant example in this context 7,8 .

An additional concern is the use of antibiotics which are causing mutations within the microbial gene pool. FAO strongly recommends against the use of such drugs because of grave future impacts. Use of such chemicals is affecting human health via chemical residues accumulated in fish. The impact has serious consequences for the future. Multiple drug resistant strains of microorganisms are evolving creating imbalance in the ecosystem. Moreover uses of antibiotics also destroy beneficial microbes which boost aquaculture performance ⁹.

Feed is of prime importance in modern aquaculture and accounts for a major share of total operational cost. Better utilization of feed directly affects profitability and environmental sustainability ¹⁰. Fish meal has historically been the protein source of choice in aquatic feeds, but global supplies have reached a plateau making it less available and more expensive ¹¹. Cost reduction of feed is a crucial aspect for the sustainability of the aquaculture sector. This can be achieved by reducing the amount of the most expensive feed constituent with an alternative ingredient without compromising on quality and yield; and poses no threat to the environment. Protein is the most expensive commodity in feed and fish meal is still the protein source held in very high regard. Fish meals have their own set of concerns: 1) disease vectors bringing infection into the culture pond, 2) stagnant global production volumes and 3) inconsistent supply based on seasonal fluctuations. Excess feeding is another issue since farmers administer large quantity to make up for the lower nutritional value of feeds ¹². In order to help the fish cultivators and address this concern, formulated feeds with minimal ecological impacts could be the mainstay.

Gradually the interest is moving towards plant based alternative protein sources. Apart from soyabean meal, floral mangrove associates native to Sundarbans such as salt marsh grass (Porteresia coarctata), and green algae (Ulva lactuca, Enteromorpha intestinalis) rich in protein and carotenoids are being used to develop meals for prawn cultivation with success ^{13, 14}. Such endeavors bring to light alternative and sustainable sources for environment friendly aquaculture. Unfortunately, the use of cheaper crude proteins from mustard cakes, dehydrated trash fish and animal entrails has flourished within the aquaculture feed industry 15, 11 which has resulted in deterioration of both fresh and brakishwater ponds leading to diseases, compromised vield and ecological damage ^{16, 17}.

Modern sustainable nutrition is been successfully used to improve immunity and help counter diseases, such as use of feed additives like -glucans from yeast to boost fish immunity reducing disease occurrence in tropical prawns^{18, 19, 20}. Immunity boosting nutrition is the buzz word, especially with the health impacts of antibiotics. Nucleotide supplementation in pacific white shrimp shows improved resistance against white spot syndrome virus, WSSV²¹. Similarly the use of alginates (from marine algae) stimulates the immune system in tropical and moderate climate fishes²². Use of probiotics is showing promising results too in improved health, growth and feed conversion ratio^{23, 24, 25}.

However not all immunostimulants can be effectively commercialized. High manufacturing costs make feed developers chose from locally available and easily sourced ingredients. Gujarat is the leading producer of commercial marine algae ²⁶ and its easy availability can assist us to formulate a combination of garlic and marine algae pure extracts. Amino acids purified from both soyabean and sea weed may be further used as a supplement.

FAO advices through technical investigations the development and implementation of amino acids, natural antibiotic-alternatives and immunostimulant based feed supplementations 15 . From 2012 – 2013 West Bengal produced 1490.01 thousand tonnes of fish, ranking them the second largest fish producing state after Andhra Pradesh²⁷. Adopting eco-friendly formulations in fishing areas of West Bengal may result in improving our fish trade. Although we can discuss at length about the advantages of alternative feed supplements, the primary challenge is to motivate the fish-farmers towards adopting such technologies. The paper provides information on the properties of amino acids, allicin and sea weed extract popular in the aquafeed trade and encouraging management action plans for sustainability in fishing areas of the Indian mangrove Sundarbans where over 70% of the population is dependent on the aquaculture trade. Awareness campaigns in the mangrove belts may assist the coastal communities to adopt more eco friendly strategies to improve fishing and sustain our environment as well as prepare us for an ever increasing demand for food. Extensive peer reviewed articles clearly indicate that amino acids, garlic and marine algae components are effective (Figure 1), and as later discussed there are a few developers pitching for the cause. The challenge is to provide support for the integration of sustainability in aquaculture programmes in ecologically critical areas of the Indian Sundarbans.

AMINO ACIDS

Amino acids can be segmented into essential, non essential and conditionally essential. The essential amino acids must be included in fish diet. The non essential ones can be synthesized by fish when required by using essential amino acids as a substrate. The conditionally essential amino acids are a branch of the non essential category, however they are included in the diet when the rate of their uptake exceeds rate of synthesis ²⁸. Amino acids control various biological processes in fish which govern their survival, development, immunity, appetite and health and their deficiency shows a lack of these. They are also documented to work as chemoattractants of feed, reduce aggressive tendencies and make them tolerant to environmental changes (salinity/temp/pH fluctuations), apart from improving taste of fish. Combinations of both essential and nonessential amino acids have been shown to possess crucial functions in fish physiology. In the current feed development industry, amino acids are seen as either a part of pro-environmental or functional feeds/supplements. The former promotes growth and immunity, while the latter makes the stock tolerant to changes in the environment 29, 30, 28. Amino acid supplementation with soya bean meal as a complete replacement of fishmeal is effective in weight gain of juvenile prawns ³¹, Nile tilapia ³², and rainbow-trout ^{33, 34} suggesting a possibility of a reduction in protein based meals for fish without compromising on growth rate and hence reducing operation costs.

Alanine and glycine have been demonstrated to improve appetite and act as chemo attractants for juvenile fishes ³⁵. Arginine has been seen to promote a variety of functions in different fishes. In channel catfish it inhibits pathogenic bacteria ³⁶; in tilapia it controls neural development ³⁷. Supplementing the diet of hybrid striped bass with either arginine or glutamine has shown to boost the immune system by increasing superoxide anion content of macrophages. It also improves growth and gut development ³⁸. A combination supplementation with arginine and histidine greatly improves growth performance in Olive flounder ³⁹. Cysteine, glutamic acid and glycine acts as antioxidants by synthesizing glutathione in all fishes ⁴⁰. Sulphur containing amino acids methionine and cysteine are crucial for gene expression and protein synthesis ⁴¹. Methionine supplemented with plant based feed exhibit desired growth in Pacific white shrimp ⁴². Glutamine has been shown to promote growth and intestine development together with improved food digestion in carp species 43. Histidine has been documented to make salmon tolerant to alterations in pH ⁴⁴ and proline promotes

growth ⁴⁵. Arginine and methionine promote growth in tiger prawns ^{46, 47}; while threonine promotes adequate weight gain in Pacific white prawn ⁴⁸. Tryptophan supplementation shows suppression of aggressive behavior in juvenile cod ⁴⁹ and improved high temperature tolerance with reduced oxygen consumption in Indian carp Cirrhinus mrigala⁵⁰. Valine supplementation has been documented to alter the gut flora composition and improve feed absorption and digestion coupled with growth in Jian carp 5^{1} . Leucine improves the immune system while lysine and methionine facilitate lipid transport in fishes ^{52, 53}. Tyrosine and phenylalanine are known to improve metamorphosis in fish larvae as they improve thyroid hormone synthesis ⁵⁴ and have been shown to promote growth in milk fish ⁵⁵. Supplementation with lysine and methionine in soyabean fed milk fish and grey mullet demonstrates optimum growth with minimal metabolic wastes ⁵⁶.

All the various amino acids have certain functions in fish physiology and they meet both functional and environmental needs of fishes. In Figure 2 we consider the various amino acids and their effectiveness in sustaining our aquaculture demands.

ALLICIN

With the growing demand for fish, the aquaculture sector is maximizing their production by using excess feed formulations with high stocking density. Such practices are resulting in immune compromised stock resulting in bacterial and fungal infections. To counter disease related loss, various antibacterial chemicals have been applied which are causing complications. Health of cultured fish is deteriorating together with a threat to the environment and humans. Bacterial antibiotic resistance is a major concern. As a result, safe performance boosting alternatives are a must. Garlic has been celebrated for centuries as a natural immune-stimulant and antimicrobial agent, dating back to ancient Egyptian medicine ⁵⁷. Garlic's zero side effects on our ecology and health makes it a choice pick for sustainable feed development.

The active ingredient of garlic is predominantly thiosulfinates called Allicin. Its broad spectrum antibacterial, antifungal and antiviral properties have been extensively documented. This includes drug resistant *E.coli* strains, protozoans and platyhelminthes which affect *Lates calcarifer* farming. This exclusive protection is attributed to its reaction to thiol groups of core biological enzymes like RNA polymerase ^{58, 59, 60}. However, researchers reveal the selective action of garlic where it acts against pathogenic variety and improves performance of beneficial ones like *Lactobacillus bifidus* ⁶¹.

Researchers ²⁴ discuss the probiotic feed benefits of this species of bacteria in aquaculture, suggesting that garlic extracts can be beneficial in enhancing such formulations. In Thailand, garlic is being mixed into feed and administered to prawn farms to avoid bacterial infections ⁶².

Together with garlic's excellent track record in controlling pathogens, it's been observed to boost growth, weight gain and blood content in fishes. Overall fish growth and haemoglobin has been documented to be significantly higher than controls ⁶³. In tilapia the supplementation of garlic has improved survival rates by stimulating a spike in monocytes. Garlic improved storage life of flesh, as it was demonstrated to last longer in ice with minimal mould infection 57. Improved quality of flesh with garlic supplementation has been attributed to an increase in protein content and lowering of lipids ⁶⁴. These effects are thought to be due to the rise in glutathione peoxidase which in turn boosts antioxidants hence protecting the tissue against oxidative radicals ⁶⁵. Sulphur containing compounds in fish impart its flavour. Investigators ⁶¹ reveal that the sulphide components of garlic when included in fish feed can improve both quality and flavour of the flesh. The garlic flavour also acts as a potent feed attractant and improves feed conversion ratio.

SEA WEED EXTRACTS

The importance of sea weeds as functional food is primarily because of their rich source of bioactive polysaccharides, antioxidants and amino acids. Apart from their use as alginate in various food grade and laboratory materials, they have a big role to play in sustainable aquafeed technology.

It is beneficial to use pure extracts rather than sea weeds themselves for the following reasons i) improve bioavailability of its active components, and ii) remove toxins present in the raw source.

Sulfated polysaccharides retard growth of pathogenic bacteria. Studies ⁶⁶ show disease resistance against strong pathogens like Aeromonas hydrophila in Pangasianodon sp. Like allicin they also act as prebiotics. The most celebrated of these are alginates, fucoidan, laminarin and galactans. These polysaccharides compose the cell wall and inter cellular matrix of seaweeds. Fucoidan is sourced from brown seaweed and possess anti viral, anti tumour, anti inflammatory and antioxidant properties. Scientists 67 have demonstrated increased survival rate of white spot syndrome virus affected tiger prawns by administering fucoidan extracts from brown or green seaweed. Laminarin is also present in brown seaweed had acts as a prebiotic, apart from containing anti viral and antibiotic properties. Nearly 47% of brown seaweed biomass is comprised of alginate and acts as a potent antibiotic and anti inflammatory. Galactans have anti tumour and antiviral activities ^{68, 69}.

Antioxidants present in seaweed are in the form of carotenoids, phycobiliproteins and polyphenols. Both of these are strong free radical scavengers with phycobiliproteins possessing additional antiviral and anti inflammatory values. Pholorotannins are a form of polyphenols which has both antioxidant and antibacterial values ^{68, 69, 22}.

Seaweeds are rich in amino acids aspartic acid and glutamic acid. In red seaweeds they comprise 14 and 19% of total amino acids. While in green seaweeds they are 26 and 32% respectively. The highest content is in brown seaweeds with 22 and 44% of total amino acids⁷⁰. Utilizing mixed sea weed extracts provide feed developers a full profile to work with and improve on the sustainability quotient.

CONCLUSION

The River Ganges flow out into the Bay of Bengal and at this confluence has formed the Indian Sundarban Mangrove ecosystem, which is an UNESCO World Heritage site for its rich biodiversity and productivity. It spans an area of 9630 sq. km and comprises of 102 islands ⁷¹. Fishing areas of Kakdwip, near the banks of the Kalnagini River (21°52'06"- 21°86'83"N, 88°11'12"E - 88°18'67"E), Namkhana (21.7667° N, 88.2333° E) and Sagar (21.8° N, 88.1° E) in South 24 Parganas, Indian Sundarbans are been extensively covered (See map in Fig. 3) in an effort to create awareness regarding sustainable aquaculture practices as a large proportion of aquafarmers are engaged in high risk intensive monoculture of tropical shrimps and prawns like Peneaus monodon and Litopenaeus vannamei. Suggested methods: (a) conducting education seminars through local Block Development Offices (BDOs) to current and prospective brackishwater cultivators as well as Government fisheries officers: (b) door to door interaction with aquafarmers; (c) distribution of free samples of supplements to aquafarmers and educating them about the benefits of using such low cost immune boosters along with sustainable practices of polyculture for tropical prawns (See Fig. 4)

The important factor is building trust, and that is done with advertising trials in experimental ponds and by providing samples to aquafarmers through seminars. A sustainable solution has meaning once visible tests are positive and farmers can 'see' the difference. Conducting seminars through Panchayat Block Development Offices is a way of breaking the ice; however it is far from melting it. The challenge is 'accepting change'. In spite of positive results local farmers are always apprehensive about trying a new solution. Hence local network with the local village development offices is a crucial step towards bridging the gap. Economic development of local fishing communities is a necessary step for overcoming their mind block on accepting a new solution. Various manufacturers have supplied spurious products with false claims that have also contributed to this hindrance ¹¹. The strength of the aquaculture community comes from the sustainability quotient. Excess feed inputs can disrupt the balance of the fish trade; however its control with designed nutrition has a promising future ⁷². Antibiotics face a strong criticism from the community, and herbal resources are still held in high regard as an alternative option. Local communities have shown considerable interest in herb based products and appreciate the need for alternative supplements which has sparked researchers to continue their investigations on herb based drugs in the feed industry ^{64, 73, 74}.

Direct farmer interactions are a must in building this trust. Door to door promotion attracts a fair response from prawn and fish cultivators of the area. Regular customer contact and feed-backs can generate 'need' based solutions. Such strategies may streamline productivity based on precise formulations as per the requirements and conditions. The manufacturer has to alter formulations for the desired result, hence creating a repertoire of feed formulations ²⁸. With a gradual shift towards plant protein sources, formulated amino acid nutritional supplementation will further the efforts in increasing efficiency in feed utilization without compromising with metabolic performances ⁷⁵.

It is imperative to improve profits for the fish farmer. Cost based analysis is an ongoing process. Pricing and logistics can prove to be a hindrance in farmer support since affordability and timely administration ensures a good crop, hence strategic locations need to be identified which can reach out to remote centres in the Indian Sundarbans⁷⁶.

A few commercial fish cultivators of the Indian Sundarbans start implementation; now the real question is whether such sustainable options will make a difference in lowering the use of excess feed, antibiotics and other potentially harmful chemicals and pave the green path towards saving our mangroves. Herbal and amino acid based feed supplement solutions are currently available to the

Indian aquafarmers like Grofast, Prolive and Biona from SRIBS Biotekno International (www.sribsbio.in); Lucanthin, Procee (contains amla extracts) and Promass from Anfomed India (http://www.anfomedindia.com/aquaculture.html); Lividol-FS-Aqua from Neospark (http://www.neospark.com/aquaculture.html). Such trends indicate a keen interest in formulators to maintain India's world ranking in aquaculture produce. However, in spite of the design and manufacturing, there is no real presence of these nutritional options in ecologically sensitive aquaculture regions like the Indian Sundarbans in spite of the very fact that eco-friendly solutions forms a stable backbone of this high demand aqua-feed industry where protein is an expensive commodity ^{74,}

⁷⁶. There is no doubt that pharmaceutical antibiotics can deliver in sprint runs, however the future remains obscured. Hence technology is gradually going back to the knowledge of the ancient Avurvedas, the Unani and Egyptian medicine of herbal uses ^{73, 74}. The World Bank survey reveals that in countries of South Asia, per capita income is on the rise, which is resulting in an increase in food fish consumption⁷⁷. Combined with new age biotechnology we can isolate purified bioactive components which are stronger and greener! Moreover, programmes for active culture of marine algae in the Sundarbans are strongly recommended. It is beneficial to use extracts rather than the plants themselves for the following reasons: i) improves bioavailability of its active components, and ii) removes any toxins present in the raw source ⁷³. With versatile functions and in therapeutic feed, sustainable applications supplementation holds considerable potential in the growth of modern day aquaculture together with protecting our coastal communities ⁷⁶.

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Fig. 1

The properties of amino acids, allicin and sea weed extracts (Li et al, 2009; Lee and Gao, 2012; Jana et al, 2012; Nwabueze, 2012; Militz et al, 2014; Chojnacka et al, 2012).



Fig. 2 Properties of amino acid in aquaculture nutrition (adapted from Li et al, 2009).



Map of the Indian Sundarbans with the major towns. Map courtesy: Indian Railways



Fig. 4

120 day culture of *Peneaus monodon* using amino acid, allicin and sea weed based feed supplementation in Kakdwip, Indian Sundarbans.

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