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EMERGING FISHERIES TECHNOLOGIES ON GROWTH ENHANCEMENT AND DIET PREPARATION

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Abstract

With the growing demand from aquaculture and foreseeable levelling off in fishmeal and fish oil production from fisheries, it appeared necessary to reduce the proportion of fishmeal in feeds for aquaculture. Research has therefore developed on other sources of protein to replace fishmeal and fish oil, in particular, using plant raw materials, while seeking to conserve the nutritional and organoleptic qualities of cultured fish. The use of vegetable ingredients in aquaculture feeds takes into account environmental consequences of the discharge of non-assimilated matter (phosphoric or nitrogenous). Phosphorous, related to phytate in the plant material, is made available by phytases, which increase its absorption and utilisation by fish though enzymatic hydrolysis, thereby decreasing phosphoric excretion (Phytate = phytic acid bound to a mineral). Phytates perform a significant role in plants, being an energy source for the sprouting seed. When a seed sprouts, phytase enzymes break down the stored phytates. After eating plant enriched in phosphorous, phytates are hydrolysed during digestion to myo-inositol-1,2,3,4,5,6-hexkisphosphate (IP6) and lower inositol polyphosphates including IP1 through IP5 (these are phytate degradation products). Research has tended to recommend adding phytases to feed formulations or choosing plant matter that is rich in phytase.

Key words: Fish meal, vegetable ingredients, Phytate, Phytase

1. Introduction

Globally there is an increasing demand for commercially valuable aquatic products. The oceans cover over 70% of the earth's surface but, even so, fourteen of the sixteen major fishing areas in the world are over-fished. Traditional approach, such as fish and seaweed farming, are helping to satisfy the demand and reduce the pressure on the wild stocks but these are restricted by natural maximum yields and the availability of suitable sites for aquaculture. Aquaculture refers to controlled farming of aquatic organisms which includes fish, molluscs, crustaceans and aquatic plants. Culture implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc., including individual or corporate ownership of the stock being cultivated. For finfish, aquaculture is analogous to the livestock and poultry industries where animals are raised in captivity and then slaughtered and sold. The application of biotechnology in aquaculture can greatly enhance the productivity of coastal areas in particular.

Aquatic Biotechnology is defined as the application of science and engineering in the direct or indirect use of aquatic organisms or parts or products of living aquatic organism in their natural or modified forms. It also includes several components like fish health and brood stock optimization, aquatic bioprocessing (obtaining valuable compounds from marine organisms), and aquatic bioremediation (use of microorganisms to degrade toxic chemicals in the aquatic environment). The short-term opportunities are primarily in aquaculture biotechnology while long term opportunities are expected in the form of marine pharmaceuticals, aquatic bioinformatics and biosensors. Biotechnology has the potential to help aquaculture enhance a cultured organism's growth rate, reproductive potential, disease resistance, and ability to endure adverse environmental conditions, such as warm or cold water. Additionally, species and serotype–specific vaccines are being developed using the techniques of modern biotechnology against the disease agents and parasites that commonly affect marine organisms.

2. Nutrition

Nutrition is an area of critical importance in aquaculture as feed represents the most significant input cost for productions amounting to approximately half of the operating expenses in intensive culture. R&D is underway using biotechnology to develop vegetable oils that mimic the characteristics of fishmeal in fish diets and to lower production costs for fish farmers. Furthermore, cultured fish that can tolerate more plant protein help reduce use of wild fish as a protein source. Product quality improvements can be achieved through the application of biotechnology to ingredient production, e.g. development of nutritionally enhanced (e.g. omega 3 fatty acid content) fish may prove to have therapeutic benefits for human health. Another potential use of modern biotechnology is the production of feeds with specific characteristics, which not only enhance the rate of growth but also influence digestibility and other desirable characteristics such as flesh colour and texture.

3. New concept on Growth Enhancement

As the cultured fish take a long time to grow to marketable size, relative to other sources of protein such as poultry, technologies that accelerate growth are important to aquaculture operators. Although not yet used commercially, growth enhancement is now possible through the application of recently developed biotechnological techniques. One such is the microinjection of growth hormone genes into fertilized eggs with proven effect of acceleration of 30-60% higher growth rate. This technology is equally effective in selfish culture practice and revolutionized the production. Growth of abalone following the introduction of growth hormone gene is being evaluated and accelerated growth open new approaches for the culture of the slow-growing mollusc. In addition, recently a marker gene is being introduced successfully for giant prawn, demonstrating feasibility of gene transfer in crustaceans, and opening the possibility of work involving gene induced economically important traits.

4. Different Type of Growth Enhancer

A. Black pepper seed as growth enhancer in *Labeo rohita*

Black pepper seed could be explored as a feed additive to enhance growth, disease resistance and survival of fish. Trial with graded levels of black pepper seed as a feed additive at 0.5% levels exhibited significantly higher SGR (Specific growth Rate; $P<0.05$), FCR (Feed Consumption Ratio). Besides muscle protein, fat, ash and fiber contents increase significantly ($P<0.05$) in fish muscle.

B. Garlic supplements

Dietary garlic has already been established as a potent growth promoter, immune system enhancer and physiological enhancer agent for fishes like C*larias gariepinus*, *Cyprinus carpio* and *Onchorhynchus mykiss*. Besides possessing various bioactive compound like Alicen, diallyl-disulfied, diallyl-trisulfied, it is also a rich source of calcium (Ca), phosphorus (P), Zinc (Zn), iron (Fe). It has a high content of carbohydrate, Silica (Si), sulfate (s) salts, BI, B complex, A and C vitamins. Application of dried garlic lobules (DGL) at 1% per kg diet significantly enhance growth, feed utilization, as also the chemical composition of muscle, hematological and biochemical parameters.

C. Fish Free Feed

Aquaculture currently uses more than 80 percent of the world's fish oil and fishmeal, which are extracted from small ocean-caught fish, leading to over-fishing of these species. According to some author (Catacutan, M.R. et al., 2014) , salmon aquaculture consumes more wild fish -- in the form of protein and oil from open-ocean fishes like mackerel, herring, anchovies and menhaden -- than it produces in the form of edible meat from farmed fish, resulting in a net removal of fish on a global basis.

Scientists have reported success in partially or totally replacing fish oil with vegetable oil in many farmed-fish species, but studies show that vegetable oil reduces the nutritional quality of the fish flesh. In contrast to vegetable oil, microalgae are much higher in essential omega-3 fatty acids, which are important for maintaining fish health and imparting neurological, cardiovascular and anti-cancer benefits to humans.

In a recent study, some researchers (Manaf, M, et al., 2014; 2015) conducted feeding study on juvenile *Nile tilapia*, a species naturally evolved to eat microalgae as part of its diet. They conducted a feeding experiment with dried Schizochytrium, a species of marine microalgae rich in health-promoting omega-3 fatty acids. The objective was to determine the optimum level of fish-oil substitution (partial or complete) for better growth of tilapia.

When fish oil is replaced totally with the microalgae, significantly higher weight gain and better food conversion were observed compared to a control diet containing fish oil, and no significant change in survival and growth rates among all diets. The fish-oil-free microalgae diet also had the highest content of omega-3 fatty acids in tilapia fillets. The study indicate that Schizochytrium can be a high-quality candidate for complete substitution of fish oil for juvenile Nile tilapia feeds, providing an innovative means to formulate and optimize the composition of feed while simultaneously raising feed efficiency of tilapia aquaculture.

The results also point to the possibility of formulating ecologically and socially sustainable aqua feeds, with greatly reduced or no fish oil from marine fisheries and without having to switch to vegetable oils from industrially farmed crops. Commercial realization of this potential will require advances in strategies to reduce non-renewable inputs, such as inorganic fertilizers and fossil fuels, and monetary costs of large-scale production of marine microalgae, as advocated by some scientists.

Feed manufacturers can explore this approach to develop aqua feeds for aqua culturists aiming to cater to the consumer willing to pay a premium for health enhanced foods. Research can be directed to find the ways to cut the high production cost. Fish oil is now totally replaced in tilapia feed and attempt is being directed towards a fish-free diet to replace fishmeal completely. Investigation are on towards finding some combinations of different marine microalgae to achieve this goal in tilapia.

D. Grape extract product touted as alternative to vitamin E in shrimp

A growth performance trial carried out by French plant extracts company, Nor Feed, in collaboration with a university at Taiwan, emphasized that a grape extract product, Nor-Grape 80, could replace vitamin E in the starter period of Pacific White shrimp production. It is claimed that vitamin replacement with the grape extract, an uncommon approach, improve the feed conversion ratio (FCR) of *Litopenaeus vannamei*, known as the Pacific white or white leg shrimp, the most widely cultured shrimp in the western hemisphere, without any mortality. Though it is not sure that Nor-Grape 80 fed shrimps would perform better than those fed the control diet with 100 ppm vitamin E, but the scientists are expecting the trial groups to be equivalent to the control group in terms of final weight or FCR, if not better. Realising the same an eight-week trial with four dietic composition including a control were conducted to evaluate the performance of Nor – Grape on fish growth as indicated below.

1. **Control group: 100 ppm vitamin E**
2. **NG 2: 0 ppm vitamin E + 50 ppm Nor -Grape**
3. **NG 3: 0 ppm vitamin E + 100 ppm Nor - Grape**
4. **NG 4: 0 ppm vitamin E + 150 PPM Nor – Grape**
5. **NG 5: 0 ppm vitamin E + 200 ppm Nor – Grape**

All the diets were composed of cellulose, binders, classic minerals and vitamin mix excluding vitamin E and prepared as cold extruded pellets having a diameter of 3 mm. Maximum growth, in terms of weight gain and FCR, were observed with group 4 (NG 4) and group 5 (NG 5) when fish were fed three times a day (Pallab K. Sarker, et al. 2016)

E. Soybean based diet to replace fish meal

Experimental trial to determine the effects of complete replacement of fishmeal (FM) by soybean on growth performance of *Carassius auratus*, common goldfish fry indicate significant improvement in growth and specific growth of fishes. Eight isocaloric (gross energy 18.27 kj g^{-1}) and isonitrogenous (crude protein 40%) diets (1–4 raw soybean based and 5–8 processed full-fat soybean based) were tried against fishmeal-based diet as control. Significant ($P < 0.01$) increase in Growth (%gain in body weight) and specific growth rate (SGR) were observed in fishes fed on soybean-based diet with a maximum increase in fishes fed on 100% processed soybean-based diet number 8. Carcass phosphorous levels were significantly ($P < 0.05$) higher in fish fed on control diet (fishmeal). Significantly ($P < 0.01$) lower levels of total ammonia excretion and reactive phosphate productions (mg kg^{-1} BW d^{-1}) along with high Food conversion ratio (FCR) were recorded when fish were fed on processed full-fat soybean compared to raw soybean diets as well as control diet. Experimental results indicate that fishmeal could be completely replaced by processed full-fat soybean in diet of gold fish and hence have positive effect on growth performance of fish.

F. Spirulina as feed ingredients

Spirulina (*Spirulina platensis*) is a blue-green vegetable micro-alga(Cyanobacteria) found in in lakes with an extremely high pH and very hard water. In some places it is being used as part of diet for human being for centuries. Like all other microbial cells, Spirulina contains all-natural vitamins including the B 'Complex range, minerals and growth factors including gram-linolenic acid (highest after milk). It contains the highest amount of β-carotene a precursor of Vitamin 'A'. It is the only vegetable source of vitamin 'B-12' containing two and half times that of liver. Spirulina is classed as a high protein diet as it consists of 60 - 71 % vegetable protein that is very easy to digest and obviously contains no fat or cholesterol that is contained in meat (Tuan, V. A., (2015). The protein content in Spirulina is three times that of soybean, five times that of meat, and the protein quality is among the best with a good degree of amino acid. The protein yield per unit area per year is the highest compared to other protein yielding crops. In Pisciculture, incorporation of spirulina in the feed for aquarium fish, Color enhancement feed for Gold fish, formulation with existing feeds for augmentation of vitamins, high protein for table variety fishes (fresh water), Special feed for shrimp farming can boost up production to manifold. It also contains essential minerals and fatty acids that will boost the immune system of the fish. Unlike other algae, it is extremely easy to digest as it is composed of soft cell walls that are made from complex sugars and protein, using these algae should prevent a lot of the digestive problems that some fish suffer with when offered other greens or other alga's

1. The benefits spirulina feeding

i) Feeding spirulina will increase and give a more uniform growth rate for fish.

Spirulina improves digestion and help providing more nutrition as it possesses no indigestible components.

ii) It aids in the prevention of swollen abdomens due to blocked intestinal passages.

iii) Spirulina enhances the production of special enzymes that break down digested fats into energy rather than letting them build up in the fishes body.

iv) It has been proven that spirulina will bring out the coloration of your fish better; this is due to the carotene pigments that are found in the algae.

Spirulina is commercially cultivated to meet the demands of the market. This is achieved by using open channel ponds with paddle wheels to agitate the water. The main spirulina farms are to be found in the United States, Thailand, India, China and Pakistan. The long-term benefits of feeding spirulina to fish is an unique way to provide nutrition to human being.

Spirulina is commercially cultivated to meet the demands of the market. This is achieved by using open channel ponds with paddle wheels to agitate the water. The main spirulina farms are to be found in the United States, Thailand, India, China and Pakistan. The long-term benefits of feeding spirulina in the fish diet has certainly passed on to the human diet, there are millions of people all over the planet who are regularly taking spirulina tablets to aid their immune systems, there is even ongoing research that it may well help control cancerous cells in the body, but obviously this still has to be proven.

G. Other plant sources

Plant-based sources include peas, lupins, corn, rice, canola, rapeseed, barley and wheat. Utilising these sources to replace fishmeal is not easy particularly in formulations for carnivorous species that require high dietary protein. Improved processing techniques enhance the nutritional contents of plant-based sources and increases utilisation as fishmeal replacement. Processing can remove ANFs and increase crude protein levels. It is argued that it is possible to substitute most, if not all, the fishmeal in practical diets with plant proteins in combination with other plant protein sources or with proteins from other sources. In some species, plant-based ingredients promote growth with high quality fillets while in others total

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fishmeal replacement is possible but with lower biological performance. One particular plant-based ingredient is rice protein concentrate, which is a good raw material due to its high crude protein (75%) and lipid (11%) content with no adverse effect on fish growth up to 20% inclusion level. However, the base material is rice and its use may not be encouraged in areas where it is a basic foodstuff of the people.

H. Terrestrial animal by-products

The good nutritional quality of terrestrial animal by-product meals makes these suitable substitutes for fishmeal. Blood meal, meat and bone meal and poultry by-product meal have been used in aquafeed production and it is estimated that quantities of these by-products are higher than fishmeal. These ingredients however, should be processed satisfactorily to prevent disease transmission. Although there has been an importation ban of these products by some countries years ago, evidence suggests that the harmful mammalian causative agent in these by-products is different from that of fish and apparently is not able to cross the intestinal barriers of fish. A good source of crude protein is meat and bone meal (50%) but the high ash content of 29% limits its use as replacement for fishmeal. An improved processing method to decrease ash level could make this material a valuable alternative protein source.

I. Non-traditional feed Ingredients

Some other sources which can be considered renewable have been evaluated as fishmeal replacement, including insects, bacterial proteins, earthworms, silk worm pupae, mopani worm, and maggot meal, among others. These have good potential as replacers for fishmeal, but there are several considerations for their usage such as production cost, acceptability by consumers, and commercial availability. Increased research efforts on these ingredients coupled with technological innovations could make these potential fishmeal substitutes important and widely used in the future. Trial on feeding experiment, indicates that inclusion of Eichhornia up to 40% significantly enhanced growth in *Cyprinus carpio*. The fish fry reared with high % of Eichhornia meal show higher feed conversion ratio (FCR) must have been due to outcome of the negative impact of the anti-nutritional factors there in proteins of plant-based ingredients. The factors like anti-nutrients and fibres have poor digestibility properties as a result these impair growth and feed utilization by fish. There is a positive correlation (r=0.625, P)

J. Microalgae - a promising feed ingredients for future aquaculture sector

Microalgae hold tremendous potential for industrial biotechnology and considered as an important resource in the production of food and medications along with many other applications. The economic use, that was difficult early, benefit ratio. Many years of research on the development of photobioreactors, which use photosynthesis to turn light energy into biomass, have yielded success. The so called "Porous Substrate Bioreactor" (PSBR), also known as the twin-layer system, based on new principle to separate the algae from a nutrient solution by means of a porous reactor surface on which the microalgae are trapped in biofilms. Special about this new procedure is that it reduces the amount of liquid needed in comparison to the currently used technology, which cultivates algae in suspensions, by a factor of up to one hundred. The PSBR procedure thus allows a significant reduction in energy and for an increase in the portfolio of algae that can be cultivated. Current successes in PSBR development and the rise in interest in this technology in recent years could signal a turn in the conception of future photobioreactors in microalgae biotechnology.

Being a traditional source of protein and carbohydrates, they can be a potent candidate as ingredients of feed industry for fish and livestock. Besides being a potent extractor of phosphorus and nitrogen from sewage and holding ability to reintroduce them into the nutrient cycle by means of organic fertilizers, can be used as a biofertilizer in profitable fish culture (https://phys.org/news).

5. Conclusion

Diet preparation of fish and crustaceans must take into account the specific requirements of individual species so that a healthy, high quality, tasty and nutritional product will come as a potential yield. It must also take into consideration the availability of the raw materials and their sustenance as a substitute (Ellis, A. E., 2001) ingredients so as to ensure the sustainability of fishery and aquaculture activities (Magnadottir, B.,2006). It is now a realized fact that nutritional approaches are essential to alleviate diseases among farmed aquatic animals5. Recently several studies put particular emphasis on immune-nutritional strategies to enlighten the importance of individual amino acids (AA) as nutraceutics for farmed fish. Glutamine, as understood, is a key source of energy for fish leucocytes6. During immune activation at cellular level, glutamine is converted in alpha-ketoglutarate that fuels the Krebs cycle, and thereby providing additional energy to sustain immune function. *In vivo* and *in vitro* experiments suggested the definitive role of arginine on immune regulation, cell proliferation and neuro-endocrine mechanisms, though its practical health implications in farmed fish are still controversial. Besides Arginine also acts as a precursor of nitric oxide, a potent bactericidal agent of the pro-inflammatory phase. Again, by being converted in ornithine it acts as a starting point for polyamine biosynthesis, an essential catalyser during cell proliferation. Tryptophan, another amino acid, is involved in immune tolerance mechanisms mediated by its metabolites, facilitating the enzymatic activity of indole amine 2,3-dioxygenase in leucocytes. The importance of tryptophan during dendritic cells activation has also been demonstrated. Methionine supplementation of feeds affected fish immune status, as observed by increased leucocyte numbers and enhanced humoral immune response. S-adenosylmethionine is an amino propyl donor during polyamine turnover and a methyl donor that might change gene expression patterns16. Methionine might also indirectly affect the antioxidant capacity by providing cysteine to glutathione synthesis. Nonetheless, few studies were centred on the exact pathways through which AA mediate their immune effects.

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