THRAUSTOCHYTRIDS IN AQUACULTURE:

CAN IT REPLACE FISH MEAL IN AQUAFEED?

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Introduction

Global fish production peaked at about 171 million tonnes in 2016, with aquaculture contributing 47 percent (80million tonnes) to the total.While capture fishery production remains static since the 1980s, aquaculture production is rapidly growing and is now almost on par with the capture fishery. The growth of the aquaculture sector is also faster than other major food production sectors registering a 5.8 percent annual growth rate during the period 2001–2016. In 2016, of the total fish production, 12 percent (about 20 million tonnes) was used for non-food purposes. A greatest part of these (74 percent or 15 million tonnes) was reduced to fishmeal and fish oil, while the rest (5 million tonnes) was largely utilized as material for direct feeding in aquaculture and raising of livestock and fur animals, in culture, as bait, in pharmaceutical uses and for ornamental purposes. About 220 aquatic animals and plants species are cultured around the world, in a vast range of production systems. Generally aquaculture production systems can be divided into feed-dependent systems or fed aquaculture (e.g. finfish and crustaceans) or non-fed aquaculture systems where culture is predominately dependent on the natural environment for food, e.g. aquatic plants and molluscs.

Significance of Fishmeal as a feed ingredient in Aquafeed

Commercial fish farms depend on aquafeeds in which fishmeal is the major ingredient. Fish meal and fish oil are profusely used in fish feed (aquafeed) for a number of reasons that include:

- Presence of high protein content, good essential amino acid profile, mineral and essential fatty acids;
- Good palatability and digestibility, thus increased growth of fish and less feed wastage; and
- Health benefits such as improved immunity, survival rate and reduced incidences of deformities.

Fish oil especially polyunsaturated fatty acids (PUFA) are considered as essential fatty acids (EFA). Docosahexaenoic acid (DHA) is required for optimal growth and fish development. In larviculture high mortality rate is mainly associated with low dietary poly unsaturated fatty acid level. Poly unsaturated fatty acids function as essential components of bio-membranes, and their levels in the tissue phospholipid fraction are associated with larval growth. DHA is relevant for the development of neural tissues such as brain and retina, considering that the larval head constitutes a significant part of the body mass, and that predatory fish larvae rely on vision to capture their food. Since fish cannot synthesize these under *de novo*, it must be obtained through diet. Wild fish obtain these omega-3 fatty acids from the fed marine algae whereas farmed fish require substantial amounts of DHA from feed.

Fishmeal/Fish oil alternatives

At present fish meal and oil is utilized and its demand is increasing worldwide. However, the limited supply and rising prices of wild harvested fishes, alternative sustainable and environmental friendly raw materials are increasingly being used in aquafeed formulations. Substantial advancement has been made to minimise utilisation of fishmeal and fish oil through the substitution of these marine raw ingredients with proteins and oils of land origin. Several attempts have been made to replace fish meal with alternate and sustainable sources, particularly plant based, which available at a reasonable price when compared to fish meal. Plant based materials also may have toxic compounds which might have negative effect on the growth of farmed fishes. Several microorganisms such as yeast, bacteria and fungi were employed to reduce the effects of anti-nutrients in plant materials and to add essential nutrients such as protein and amino acids.Protein derived from crops plants used in aquafeed have low digestibility and lack certain essential amino acids such as lysine, methionine, threonine and tryptophan. The use of oils of plant origin as a substitute for fish oil has significantly increased.However, replacing fish oil with vegetable oil has many limitations. Since these oils are rich in n-6 fatty acid, inclusion with such oil decreases n-3:n-6 ratios in farmed fishes. Moreover longer chain fatty acids are more beneficial than shorter chain fatty acids. Hence it is important to find out alternative sources of proteins and lipids which are economical and sustainable.

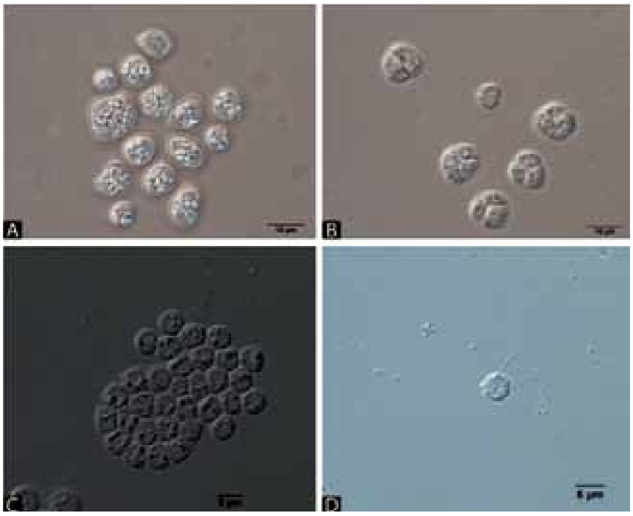
Microalgae as aquafeed ingredient

Microalgae represent one such feed ingredient with superior nutritional quality and potential availability. Amino acid profile of numerous algae observed contain all the essential amino acids even if the total protein content (8–50% dry weight) shows significant variations. In addition to that almost all algae are also rich in n-3 LC-PUFA. Thus algae or algae derived products appear to be a very promising raw material for use in aquafeed. Algae are the primary producer in aquatic food web. Currently more studies are focussing on the utilisation of algae as a potential ingredient for aquafeed development. Several studies around the world suggested that incorporation of algae (2.5–10% of the diet) in fish feed resulted in positive effects. Algae meal act as a good feed supplement for neutralising intestinal inflammation and also aid in aquafeed pelletisation. It was documented that the dose and species influences the effect of algal inclusion in feed. Even so, high inclusion level of algae in aqua feed has adverse effect on fish growth and feed efficiency. Both autotrophic and heterotrophic algae are used for replacement study. Studies on autotrophic algae Ulva spp. showed that inclusion of at various levels in feed, exhibited a reduction in growth and feed utilisation in fishes rainbow trout fed with 10% algal meal. Such results are also observed in black sea bream and gilthead sea bream (15%), and common carp and Nile tilapia(20%). These harmful effects may be due to the presence of anti-nutritional compounds like lectins, tannins, phytic acid, and protease and amylase inhibitors present in algae. When used at a low inclusion algae are very useful ingredients for aquafeed fortification.

Among variety of microalgae, autotrophic microalgae have several disadvantages with commercial production, whereas heterotrophic microalgae act as a promising candidate with significant long chain ω-3 fatty acid production potential and hence heterotrophic microbial research has gained extensive interest for the past decades.

Thraustochytrids - A potent feed ingredient

Thraustochytrids are one of the potent organisms in PUFA market. Thraustochytrids are osmo heterotrophic, oleaginous, eukaryotic, unicellular monocentric fungi like Protista. They are classified under kingdom Heterokonta and class Labyrinthulomycetes (Leader et al. 2004 , Damare, 2009). Thraustochytrids comprise 10 genera (Yokoyama and Honda, 2007, Raghukumar



Microscopic image of Aurantiochytrium sp. A. Thallus B. Mitotic cell division in thallus, C. Sporangium and zoospore release, D. Zoospore

2011) comprising more than 30 species. On an average Thraustochytrids under non optimised condition produce total fat of about 10–50% of biomass and 30–70% of which is DHA. The production capability of Thraustochytrids for biomass, total lipids, and DHA content vary largely. The production of lipid peak mostly at the end of the exponential or the early stationary phase and is greatly influenced by medium composition, incubation temperature, pH, culture age, seawater concentration, and impeller speed and shape in fermenters. Apart from these physico- chemical parametres, selection of suitable strain is also important to get good yield.

Over the past several years research activities were focussed to optimise various physico-chemical factors to enhance DHA productivity. The optimum salinity and temperature required for biomass and fatty acid production is 15-22.5‰ and 20-25°C respectively. Thus standardisation of culture condition is required to develop maximum biomass in a relatively short time span. For example *Schizochytrium limacinum*, originally described species, SR21 produce, a lipid of about 50% of the dry cell weight with 93% triacylglycerol (TG). DHA content of the lipid was 34% of total fatty acids. Detailed studies on this strain yielded maximal biomass values of 48.1 g biomass L−1 medium in 4 days, up to 77% of lipids in biomass and 43.1% of DHA in lipids, amounting to 13.3 g L−1 medium.

To replace fish oil several fish feeding strategies using thraustochytrids are attempted. One strategy is by enriching larval brine shrimps or rotifers with live thraustochytrid cells prior to feeding them to fish. The second strategy employed direct feeding of thraustochytrids (spray dried or freeze dried mix pellet) to fish or molluscs. A third strategy involves the formulation of a fishfeed comprising of thraustochytrid derived oil or meal as an ingredient in the recipe. Several of such studies on fishes confirm that thraustochytrid oil can be used as an adequate alternative to fish oil.

The incorporation of dried *Schizochytrium* strain in the diet of channel catfish (*Ictalurus punctatus*), at low concentrations of 1.0–1.5%, lead to an increased weight gain along with improved feed efficiency ratio and levels of PUFA. Another experiments including spray-dried biomass of *Schizochytrium sp*. at 5% in feeds for Atlantic salmon successfully replaced fish oil without affecting fish growth rate and food conversion ratio (FCR), dietary protein, energy digestibility, and flesh quality. Similar fish oil replacement trials done in tilapia showed significantly improved weight gain, feed conversion ratio, and protein efficiency ratio and also had higher content of DHA in the fillet lipids, which reflect higher DHA supplied through the diet. It was also concluded that in Pacific white shrimp (*L. vannamei*) larvae *Schizochytrium* (4%) meal incorporated feed can improve growth performance. It has been observed that heterotrophic microalgae *Schizochytrium* are promising due to their nutritional quality and ability to be efficiently utilized by the desired aquaculture species. Moreover mass cultivation technology of *Schizochytrium* is fully developed, and can be produced commercially to reduce the use of fish oil in aquafeed in the near future.

Commercial applications of Thraustochytrids

Several product based on Thraustochytrids are available now. Several countries around the world now are producing omega-3 rich oils from thraustochytrids as possible improved or alternative sources of PUFA. DSM and Alltech are such companies. While DSM is the leader in the production of omega-3 rich oil from thraustochytrids (DHAgold™) for human consumption and animal feeds, Alltech is targeting animal feeds particularly with their product All-GRich.