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# Status of aquaculture with respect to nutrition and feed

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**Abstract**
Aquaculture is basically rearing of aquatic animals, majorly fishes. Fish is very important animals and considered as a poor man protein food due to its lesser price. Fish consumers are greatly increasing with the current annual per capita consumption of 20/Kg. From the past 50 years, around 90% of the fish populations were destroyed by commercial fishing as fishermen use massive ships, innovative electronic equipment and satellites to find the fish for fishing. It is imperative to note that some areas due to low catch, fishermen are finding alternate jobs for their livelihood. According to FAO, there was a steep growth in the aquaculture industry for the last seven decades as the dependence of capture fishery for fish has greatly reduced. In that sense, only by capture fishery it is really difficult to meet the protein requirement to the human population; thus, aquaculture is now playing a substantial role. In aquaculture, profitability relies on the production of healthy fish which require quality ingredients and diets. Also, the performance and growth of aquaculture industry will greatly affect by feed costs as they constitutes around 50 to 60% of the total operating cost and therefore, fish nutrition and feed research are expected from these areas. The nutritional research encompasses the study of nutrients necessary for the maintenance of life. While the feed research covers the study on the preparation of quality ingredients which yield nutrients needed for the growth and performances of the animals. At present, works on fish feed nutrition is concentrated on the following areas: the nutrient requirements for larval, adult and broodstock fishes; metabolic role of each nutrients on growth and reproduction; manufacturing of artificial feed using low cost unconventional feedstuffs replacing with costliest conventional feed ingredients; identification of feed additives to improve the metabolism and growth; formulation of the standard feeds using quality and low polluting nutrients. The present paper discusses the challenges and scope of aquaculture industry in relation to nutrition and feed; but special attention has given to the aforementioned areas.

**Keywords:** Aquaculture, Nutrition, Feed, Current status, Future need

## 1. Introduction
Ancient aquaculture practices were greatly relied on extensive type of fish rearing, which involved less stocking density and no artificial feeding. Later stages it came to semi-intensive farming which need artificial feeds along with natural feeds for the growth and survival of animals as the stocking density is moderately high as compare to extensive rearing system (Halver and Hardy, 20012) [1]. Now it is the practice of rearing the fish in the intensive farming practices where, artificial feeds are the major source of nutrients for the animals reared in this system as animals are high stocked (Stickney and Treece, 2012) [2]. The animals reared in the intensive systems need more oxygen, nutrients and feed for the growth and survival (Shepherd and Bromage, 1988) [3]. As stated earlier, in the ancient days the dependence of artificial feeds are less common and farmers formulate their own feed for the animals. Recently, the augmentation of semi-intensive and intensive farming of fishes increased the demand for the feed (Tacon, 1995) [4]. In this context, farmers wish to use cost less artificial feed which can reflect in their economy. But the gradual increase for the price of conventional feed ingredients used in the fish feeds disturbs feed formulators to prepare cost less feeds (Daniel, 2016) [5]. This will be a real threat to the fish farmers if it is not properly addressed. During the 1960s, the price of the feed was significantly lower than that of current price. Due to the occurrence of situations discussed above, many small scale aqua farmers are formulating their own feeds. The farmers have less knowledge and facility for the preparation of quality feeds and have no idea of supplementing the necessary additives in the feed. In order to help poor farmers, the significant input is needed from fish nutritionists and farmers must also learn new strategies of how best they can reduce the feed costs. To achieve these goals prominently, at least in the future many potential unconventional costless feed ingredients must be identified for the

reduction of feed costs.

Despite fish culture started several centuries ago, the systematic and scientific study of fish nutrition started only at the eighteen century. Then it gradually attains all the necessary information related to nutrition of fish (i.e. structure, function and requirement of the nutrients needed). But it got considerable growth only in the middle of the twentieth century. However, the information regarding fish nutrition is still lagged behind than that of humans and terrestrial animals (Jobling, 2016) [6]. The research on fish is difficult than that of humans and terrestrial animals as maintaining them in the in the captive and controlled environment is not as easy as compared to humans and terrestrial animals (McLarney, 2013) [7]. This is a one major reason why considerably less and only narrow ranges of studies have been carried out in the fish nutrition at the past. However, vast and extensive researches have been carried out in the last decades in the different fields of fish research. Besides, the sharing of research ideas and the publication of research got very good interest among the researchers in the last decades. This article reviewed few recent publications in the aquaculture field in relation to nutrition and feed research. This review transfers the information of what is expected from the fish feed and nutrition research in the future and what areas need immediate focus and how they should be addressed for the sustainable growth of aquaculture and aquafeed industries.

**Scope of feed industries in human nutrition**

Today the number of human beings exists in the world is 7.4 billion. It is projected that at 2024 it may reach 8 billion and at 2056 it may reach 10 billion (worldometers.info) [8]. Among that 795 million people or every one in nine are suffering from hunger and malnutrition (worldhunger.org) [9]. According to United Nations Food and Agriculture Organization (FAO, 2008) [10], it was predicted that in the 2050 the demand for food will increase to 60%. According to feed industry federation, in order to tackle these it was assumed that meat production, dairy and fish sectors will contribute 70%, 55% and 90% at an expected annual growth rate of 1.7% (ifif.org) [11]. At present, around 70 billion animals are farmed for food purposes, including fish and by 2050 it will be twice higher than that of present (worldanimalprotection.org) [12]. According to awell-fedworld, animals consumed by humans are more than 30% is now coming from the farm raised (awfw.org/factory) [13].

**Comparison of animal feed production with aquafeed production**

Over the last few years, both animal and feed production got improved growth rates. In the year 2006, the global feed production was 25.4 million tonnes (Gill, 2007) [14]. The global feed production at the 2011 was 873 million tonnes which valued for $350 billion (Alltech, 2015) [15]. But now it increased. At present, there are 31,043 feed mills are existing in the world which make 980 million tonnes of feeds which is valued at $460 billion (Coffey *et al*., 2016) [16]. However, now the challenges are raised in the growth of feed industry mainly due to the occurrence of high costs of raw materials, fluctuation in the trend of import/export policy of government (Alltech, 2014) [17]. According to International feed industry federation annual report 2014-15, China produces 19% of the total feed, followed by USA 17%, EU 16%, Brazil 7% and rest of the world 41%. The sector wise, poultry sector produces 45% of the total feed, followed by pig-26%, ruminant-20%, aquaculture-4% and the rest is 5% (annualreport. ifif) [18]. This indicates aquafeed sector has a dormant stage, need outstanding growth to play a role for the sustainable fish production. Report says the fish meal and fish oil production had a slow growth rate stage for the last 25 years. During 1977, the global fishmeal production was 4.57 million tonnes, but in 1994 it reached only 7.48 million tonnes. Similarly, the global fish oil production was 1.67 million tonnes in the year 1986, but in 2002 it has reduced to 0.85 million tonnes (Tacon *et al*., 2006) [19]. Due to the less production of fish meal, now poultry and pig sectors uses only 25% of fish meal, followed by ruminants use only one % and the rest 70% is solely used by aquafeed sector [10]. The consumption of fish meal and fish oil increased three fold in the aquafeed for the last few years (Tacon *et al*., 2006; Tacon, 2007) [19, 20]. In 2006, the amount of fish meal and fish oil used in the aquafeed was 3.06 million tonnes or 56.0% and 0.78 million tonnes or 87.0% to total feed production, respectively (Tacon, 2007) [20]. But now production of low valued fish from capture fishery is getting down due to many reasons, aquafeed sectors as well relies on the alternate feed ingredients to replace the fish meal and fish oil for feed production.

**Areas covered under aquaculture in relation to fish nutrition and feed research**

The critical need of nutrition and feed research to the aquaculture industry is greatly agreed by many workers. The works attempted in these fields are highly diversified with different areas of aquaculture in relation to nutrition and feed research, which is covered in this review.

**Carbohydrate nutrition**

Carbohydrate is the cheapest food source for the human beings, animals as well as aquatic living beings (Lavell, 1989) [21]. It gives an energy value of 7.2 kilojoule/gram (Blaxter, 1989) [22]. It is also used as a binder in fish feed. The carbohydrate utilization by fish is varies to different species (Buddington and Christofferson, 1985) [23]. Fish is considered as the diabetic animals which don't prefer energy source from it as feeding more carbohydrate will create hyperglycaemic condition which results in the metabolic stress to animals (Wilson, 1994) [24]. It was believed that most fish found in the wild are predominantly either carnivorous or omnivorous feeding habits, fish didn't practice to eat carbohydrate rich foods and hence, carbohydrate rich food is not a major choice of food for them. It was also hypothesized that fish evolved in the environment contain an abundant sources of proteins, which make animals to favourably prefer protein rich feeds and resist to carbohydrate rich feeding. It is worth noting that feeding protein rich food to fish is economically unsound to the farmers; therefore they wish to go with cheaper alternative carbohydrate sources (Stone, 2003) [25]. Carbohydrate also has protein sparing effect (Wilson, 1989) [26], but high intake level gives deleterious effect to fish health. In addition, carbohydrate utilization is less in fish compare to higher animals as stated before, (Jauncey, 1982) [27]. It is reported that high carbohydrate in the diet leads to liver damage of fish (Hess, 1935) [28]. It is also found that even after the digestion, glucose is not used for the energy by fish; sometimes it is lost through the urine (Bureau *et al*., 1998) [29]. High glucose level in the diet will increase the oxygen consumption of fish when replace with protein (Beamish *et al.*, 1986) [30]. High glucose intake also lowers the protein retention efficiency of fish. The addition of complex carbohydrates in the diet, these problem can only be little resolved as the slight increase of glucose levels offer metabolic stress to the fish. Therefore, the optimum level of carbohydrate in the fish feed is very essential (Beamish *et al.,* 1986) [30].

Various information is available regarding the topic on fish carbohydrate nutrition suggesting that it is possible for herbivorous and omnivorous feeding habituated animals to expand the level of carbohydrate in the diet at the maximum levels; and this is debate to the carnivorous fish as they preferentially wish to take animal originated protein feeds (Shiau, 1997) [31]. Besides, herbivorous and omnivorous fish has enzymes for the digestion of carbohydrates and these enzymes are lacking in the carnivorous fish. There were many potential animal based feeding stimulants as well as exogenous cellulose, amylase or some carbohydrases were identified to improve the carbohydrate utilization to the animals (Xie *et al*., 2016) [32]. These strategies can be followed to expand the level of carbohydrate in the fish diets. Though this strategy can be adopted, the dosage must be standardised and documented for the cultivable species as fish is species specific in nature. This documentation will help in the preparation of cost effective diets with the maximum probable levels of carbohydrate in the feed for fish.

**Protein nutrition**

Protein is the necessary component in the diet of all living beings, including fish (Cho and Kaushik, 1990) [33]. It is a vital component for the animal's survival. Protein is the foundation for tissue repair, balance and tissue formation; it constitutes around 65 to 75% of the total body weight on dry matter basis. One gram of protein contains energy values of 23.6 kilojoule (Blaxter, 1989) [22]. The protein requirement for fish is significantly higher than that of human and the terrestrial animals. It is established that fish need two to four folds in the diet as compare to terrestrial animals. Speaking scientifically, any animal as such does not need protein in the diet; they need balanced amounts of amino acids called essential amino acids (EAA) (Halver and Hardy, 2002) [1]. EAA can't be synthesized by fish *de novo* and they must be present in the diet of the animals (Kaushik and Seiliez, 2010) [34]. But fish can able to synthesize some amino acids by their own is known as non-essential Amino acids (NEAA) and they are not necessary to be present in the diet (Li *et al*., 2009) [35]. Therefore, development of fish diet balanced with essential amino acids is very important (De Silva and Anderson, 1994) [36].

The costs of protein ingredients are significantly higher. Therefore, making fish to draw energy from carbohydrates and lipids and make sure the proteins only available for growth would be of great profit for fish farmers (Murrai *et al*., 1985) [37]. The protein ingredients contain relatively high amounts of AA profile have high biological values (BV). It is suggested that ingredients in the diet contain more BV chances to improve the protein accretion in fish. It is found that less protein in the diet mobilizes the body protein for maintenance and lessens the weight of the fish. If more protein is added in the diet, only the required amounts of protein can be utilized by the animals and rest will be converted into energy, in turn improve feed costs without need. Therefore, the balanced AAs along with, proper digestible protein-to-digestible energy ratio and high BV ingredients will ensure maximum protein deposition of fish (Halver and Hardy, 2002) [1].

Fish meal is the major conventional protein ingredient in the fish feed, but due to its high cost now feed is prepared with lesser incorporation of it. Currently, the majority of researchers involved in the protein nutrition are studying on finding out the other unconventional cost less protein ingredients for the aquafeed usages. However, replacing them with conventional feed ingredients have demerits that they are less in some EAA as well as contains anti-nutritional factors (ANFs) which hinders the animals for the utilization (Francis *et al*., 2001) [38]. An addition of proteases enzymes in the fish feed could be followed to improve the digestion as well as the removal of ANFs. Certain unconventional feed ingredients have significant protein percentage and good amino acid profiles, except one or two amino acids lacking in them. In these conditions, the supplementation of the amino acids which is deficient would be a best solution. It was also reported that certain amino acids in the diet can improve the bio-availability of other EAAs. Liang *et al*. (2016) [39] showed that Blunt snout bream fed with 1.96% of dietary arginine in the diet lowers the plasma lysine levels and increase the plasma histidine, isoleucine, valine, tryptophan and methionine levels. Therefore, the addition of deficient amino acids along with balanced amino acid content in the diet is necessary for the preparation of cost effective commercial diets (Wang *et al*., 2016) [40].

**Lipid nutrition**

Lipids are the major source of metabolic energy for growth and reproduction in fish (Henderson *et al*., 1984; Wilson, 1989; Sargent *et al*., 1989) [41, 26, 42]. It contains the energy values of 39.5 kilojoule per gram (Blaxter, 1989) [22] which are higher than carbohydrate (17.2 kilojoule/gram) and protein (23.6 kilojoule/gram). Lipid contains omega 3 fatty acids, especially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linolenic acid and omega 6 fatty acids such as arachidonic acid (ARA), linoleic acid which are rich in energy content and vital for many necessary physiological functions of the animals (Henderson and Almater, 1989) [44]. They also contain cholesterol and phospholipids which are needed for fish and shrimps in all stages of the development. As protein is costly, often proteins are spared by either lipid or carbohydrates; i.e. digested proteins are metabolized for growth and energy are provided by either lipid for carnivorous fish or carbohydrate for herbivorous/omnivorous fish. Previous studies showed that sometimes proteins oxidised to amino acids will be directly used as energy through tri-carboxylic cycle or sometimes they are converted into glucose via process called gluconeogenesis (Halver and Hardy, 2002) [1]. However, this can be greatly minimized through supply of abundant protein sparing energy nutrients. In general, provision of 10-20% of lipid in the feed (dry weight basis) one can make sure that greater portion of protein is used by fish for growth without generating fatty fish (Watanabe, 1982) [43]. Lipids are the major source of energy particularly for carnivorous feed, because they have very limited potential to use carbohydrate feed due to the fact that the availability of carbohydrate food is less in the natural waters they evolved and hence, they habitualized to grab more energy from the lipids. In contrast, herbivorous and omnivorous fishes adapted to take more energy from the carbohydrates and less from lipids, because they have enzymes for the digestion and utilization of carbohydrates (Watanabe, 1982; Smith, 1989) [43, 45].

The feed ingredients often used in the feed are less of omega 3 fatty acids. In addition, fish does not have enzymes for the synthesis of omega 3 fatty acids. Therefore, the supplementation of omega 3 fatty acids to the diet is crucial for fish. The metabolism of lipids in fish is the results of different dietary levels of lipid intake. It also varies depends on the presence of saturated or unsaturated fatty acids in the diets. For the adaptation to low lipid intake fish induces, glycolysis, lipogenesis and depress lipolysis. For the adaptation to high lipid intake fish reduce lipogenesis and induce β-oxidative and gluconeogenesis (Halver and Hardy, 2002) [1]. Fatty acids and glucose play an important role in the appetite systems of fish by dietary lipid modulation via leptin expression (Li *et al*., 2016) [46]. The adaptation and maintenance of animal's membrane lipids and fluidity to the different temperature is called homeoviscous adaptation. This is due to lipid content in the body. It is observed that animals receive more omega 3 fatty acids in the diet able to withstand to the sudden temperature fluctuations often arise in the pond environment (Portilla *et al*., 2016) [47]. The requirements of fatty acids are specific to fish species (Sales, 2003) [48]. In general, fresh water fishes often require linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) in the diet; while marine water fishes require eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) in the diet (N.R.C, 1993) [49]. Previous studies in the Japanese seabass reported that they can able to utilize high levels of C18:1n-9 and C18:3n-3 in the diet, but they can't utilize high levels of C16:0 or C18:0 in the diet which results in the lower feed efficiency and growth. Therefore, studying different lipid sources in the diet will help in the preparation of diet with balance fatty acids and lipid profiles (Xu *et al*., 2016) [50].

The imbalance of fatty acids in the fish also imparts negative impact to the animals. Increase lipid intake of fish generate oily fish, oily texture, which cause poor smoking and rancidity problems. Beside, more lipids in the feed require more amount of costliest antioxidants in the feed as lipids are easily oxidised (Hemre and Sandnes, 1999) [51]. Besides, oily fish reduce the coloration by minimize the carotenoid and other pigments which consequently leads to poor preference by consumers and less marketability (Bell *et al*., 1998; Hillestad *et al*., 1998; Refsgaard *et al*., 1998) [52,53,54]. Therefore, it is advised that the supplementation of lipid in the fish diet, one must consider the all above discussed factors.

## Vitamin nutrition

Vitamins are essential element to all the animals for their physiological functions. It is vital component for the many proteins, enzyme and hormones. They come under micronutrients as they are need in micrograms. But its absence in the diet make animals vulnerable to many diseases (Lavell, 1989) [21]. However it is true, animals develop to disease only when they eat vitamin deficient diets for a long interval of time (Halver and Hardy, 2002) [1]. Many vitamins are acting as coenzymes for many enzymes (Sandnes, 1991) [55]. Many diseases encountered in the farming animals are often connected with vitamin deficiency in their diet (Halver, 2002) [56]. There are mainly two types of vitamins; water soluble (Thiamin, Riboflavin, Pyridoxine, Pantothenic acid, Inositol, Biotin, Folic acid, Choline, Nicotinic acid, Ascorbic acid and p-Aminobenzoic acid), which are soluble in the water and fat soluble (Vitamin A, D, E and K), which are soluble in fat. Fish mostly require all vitamins in the diet. Most of the vitamins are unstable to the heat, temperature, light or processing in their nature forms. Therefore, they often need more than the animals requirement in the diet. But this is effectively remedied by the binding of unstable vitamins with the other stable compounds. By doing so, unstable forms of vitamins can be protected from such conditions and can be easily obtained by the fish.

Fish diet is prepared mostly from agricultural by-products they are less in vitamins. But other ingredients used to formulate feed contain some amounts of vitamins in it; still they are lesser than the animal requirement (Halver and Hardy, 2002) [1]. The vitamin deficiency (Avitaminosis) occurs mostly due to deficient in water soluble vitamins; because water soluble vitamins are quickly utilised and absorbed by the animals. Avitaminosis in fish due to lack of fat-soluble vitamins are not common; because they metabolize slowly and often they stored in the tissues. However, when animals receive very less fat-soluble vitamins in the diet or if animals grow very fast and get less fat-soluble vitamins in their diet, avitaminosis is possible. In contrast to avitaminosis conditions, hyper-vitaminosis also recorded in the fish when they receive more fat soluble vitamins such as vitamin A and D in the diet (Burrows *et al*., 1952) [57]. Hypervitaminosis causes problems such as, hepatocyte destruction and liver necrosis to the animals. But larger fish take more time to develop vitamin deficiency symptoms as compare to smaller fishes, because larval fishes need them more during the developmental stages and survival (Hardy, 2001) [58]. The deficiency of vitamins in the diet cause various diseases to the aquatic animals, listed in the table 1.

**Table 1:** Vitamin Deficiency syndrome in fish

| Vitamin | Deficiency syndrome |
|---|---|
| Thiamin | Poor appetite, muscle atrophy, convulsions, instability and loss of equilibrium, edema, poor growth. |
| Riboflavin | Corneal vascularization, cloudy lens, hemorrhagic eyes, photophobia, dim vision, incoordination, abnormal pigmentation of iris, striated constrictions of abdominal wall, dark coloration, poor appetite, anemia, poor growth. |
| Pyridoxine | Nervous disorders, epileptiform fits, hyperirritability, ataxia, anemia, loss of appetite, edema of peritoneal cavity, colorless serous fluid, rapid postmortem rigor mortus, rapid and gasping breathing, flexing of opercles. |
| Pantothenic acid | Clubbed gills, prostration, loss of appetite, necrosis and scarring, cellular atrophy, gill exudate, sluggishness, poor growth. |
| Inositol | Poor growth, distended stomach, increased gastric emptying time, skin lesions. |
| Biotin | Loss of appetite, lesions in colon, skin coloration, muscle atrophy, spastic convulsions, fragmentation of erythrocytes, skin lesions, poor growth. |
| Folic acid | Poor growth, lethargy, fragility of caudal fin, dark coloration, macrocytic anemia. |
| Choline | Poor growth, poor food conversion, hemorrhagic kidney and intestine. |
| Nicotinic acid | Loss of appetite, lesions in colon, jerky or difficult motion, weakness, edema of stomach and colon, muscle spasm while resting, poor growth. |
| B12 | Poor appetite, low hemoglobin, fragmentation of erythrocytes, macrocytic anemia. |

| | |
|---|---|
| Ascorbic acid | Scoliosis, lordosis, impaired collagen formation, altered cartilage, eye lesions, hemorrhagic skin, liver, kidney, intestine, and muscle. |
| p -Aminobenzoic acid | No abnormal indication in growth, appetite, mortality. |
| A | Impaired growth, exophthalmos, eye lens displacement, edema, ascites, depigmentation, corneal thinning and expansion, degeneration of retina. |
| D | Poor growth, tetany of white skeletal muscle, impaired calcium homeostasis. |
| E | Reduced survival, poor growth, anemia, ascites, immature erythrocytes, variable-sized erythrocytes, erythrocyte fragility and fragmentation, nutritional muscular dystrophy, elevated body water. |
| K | Prolonged blood clotting, anemia, lipid peroxidation, reduced haematocrit. |

Adapted from (Halver and Hardy, 2002) [1]

## Mineral Nutrition

The minerals are important element required for the animal physiology (Watanabe *et al*., 1997) [59]. They are part of many enzymes, hormones and proteins (Fotedar *et al*., 2016) [60]. The minerals linked in the formation of enzymes necessary for the biological function is given in the table 2. They are also necessary for the formation of bones, regulation of pH and the maintenance of normal health physiology (Lall, 2002) [61]. As like vitamins, most minerals are required in micro grams; hence, they comes under micronutrients. Fish need this for the growth; prawns often require this for moulting. The deficiency of minerals often leads to low survival in the larvae and less reproductive success for the brooders. In order to improve the pond fertile, certain minerals are necessary. But the capacity of minerals derived from the animals is different based on the living conditions. A marine water fish has certain advantage to get some minerals through water. In other hand, fresh water has some benefits to acquire certain minerals through water. Therefore, the requirement of minerals vary among for freshwater and marine water fishes as both systems have rich or less in some essential minerals in the sufficient amounts. In the laboratory studies, different disease has been recorded due to the mineral deficiency. In some case mineral toxicity also recorded with high intake of certain minerals, including zinc, selenium, etc.

By considering the cost increment of animal ingredients, the expansion of plant based ingredients to the diet got real interest today. However, plant based proteins have anti-nutrients which often make selenium unavailable to the animals. It was reported that through the supplementation organic selenium in the diet, one can improve the amount of plant based ingredients in the fish diet (Fotedar *et al*., 2016) [60]. Certain minerals have role in the strengthening of immunity (selenium) and reproduction (zinc and manganese) in fish. Lack of certain minerals (selenium) in the diet also lowers the antioxidant capacity of animals. Certain minerals such as copper have also role in the proper haematology, metabolism and stress (Wang *et al*., 2016; Damasceno *et al*., 2016) [62, 63]. Therefore, the requirement study for minerals in fish must consider their need not only for growth; also for other parameters. The focus on the mineral nutrition must pay attention in the areas discussed above.

**Table 2:** Minerals which are part of enzymes necessary for biological function in fish

| Mineral | Enzyme | Function |
|---|---|---|
| Iron | Succinate dehydrogenase<br>Cytochromes (a, b, c)<br>Catalase | Aerobic oxidation of carbohydrates.<br>Cytochromes (a, b, c) Electron transfer.<br>Catalase Protection against $H_2O_2$. |
| Copper | Cytochrome oxidase<br>Lysyl oxidase<br>Ceruloplasmin (ferroxidase)<br>Superoxide dismutase | Terminal oxidase.<br>Lysine oxidation.<br>Iron utilization, copper transport.<br>Dismutation of the superoxide free radical ($O^{2\cdot-}$). |
| Zinc | Carbonic anhydrase<br>Alcohol dehydrogenase<br>Carboxypeptidases<br>Alkaline phosphatase<br>Polymerases<br>Collegenase | CO2 formation.<br>Alcohol metabolism.<br>Protein digestion.<br>Hydrolysis of phosphate esters.<br>Synthesis of RNA and DNA chains.<br>Wound healing. |
| Manganese | Pyruvate carboxylase<br>Superoxide dismutase | Pyruvate metabolism.<br>Dismutation of the superoxide free radical ($O^{2\cdot-}$). |
| Molybdenum | Glycosyl aminotransferases<br>Xanthine dehydrogenase<br>Sulfite oxidase<br>Aldehyde oxidase | Proteoglycan synthesis.<br>Purine metabolism.<br>Sulfite oxidation.<br>Purine metabolism. |
| Selenium | Glutathione peroxidase<br>Type I and III deiodinases | Removal of $H_2O_2$.<br>Conversion of thyroxide to the active form. |

Adapted from (Halver and Hardy, 2002) [1]

## Larval nutrition

The early development stage is very crucial for the aquatic animals. During this stage, the mortality percentage of animals is high and they must get proper nutrition for the normal growth, physiology and survival (Rønnestad, 1999) [64]. In the artificial ponds or tanks natural feed (live feed) is less, often animal requires artificial feed. The larvae require more protein, amino acids, vitamins, minerals, lipids, fatty acids in the diet (Infante and Cahu, 2001) [65] often increase the feed cost. For larvae, optimization of nutrients, their requirements and feeding time are very crucial. The mouth size of the larvae is very small; as a result, they need small sized pellet which can be consumed by them. The development for the artificial feed for the larvae is still not yet properly standardized as they are not same that of adult fishes. Therefore, this area needs significant input from aquaculture nutritionists. It is speculated that changing the feeding regimes will change the production of digestive enzymes for the digestion. But the study in gilthead sea bream larvae showed that changing the feeding regimes does not change the production of digestive enzymes (Zeytin *et al*., 2016) [66]. Larvae have limited amounts of digestive enzymes. Therefore, addition of digestive enzymes in the feed would improve the digestibility and utilization of feed to the fish larvae.

Nutritional programming of the early larval fishes is the recent interest in area of larval nutrition. It is true that nutrition of early larval diet will alter the life changes in the later stages (Fang, 2014) [67]. Larval feeding will shape the later stages. Olden days, the study on larval fish nutrition carried out mostly in the study of myogenesis or muscle growth during the development with the intrinsic factors such as genotype, extrinsic factors like photoperiod and temperature. But now it is directed towards the nutritional factors such as supplementation of vital nutrients in the diet of larval feeds. It is believed that feeding vital nutrients during the early development stage will have significant effect in the later stage of the animals (Canada *et al*., 2016) [68]. In the earlier study, it found that supplementing the crystalline indispensable amino-acids to the microdiet improved the growth of muscle and related genes expression in Senegalese sole larvae (Canada *et al*., 2016) [68]. This is the relatively new area in the larval fish nutrition, scarcely studied; must be copiously explored and documented.

**Broodstock nutrition**

The research on broodstock nutrition is the key areas for the many researches as healthy parents can assure the production of healthy young ones (Izquierdo *et al*., 2001) [69]. There are varieties of nutrients that are required for the healthy broodstock. It was understood that the broodstock receiving healthy diets produce healthy young ones with more survival rates. Broodstock feeds are costly as they must be enriched with high proteins, omega 3 fatty acids and other essential micronutrients (Dabrowski and Ciereszko, 2001) [70]. In the hatchery microalgal diets are commonly used as they rich in essential nutrients required for the breeding and hatching. The study from the Geng *et al*. (2016) [71] suggested that compositions of fatty acid and sterol can be either fed with single and mixed microalgae, which would be best feed for the brooder fish to minimize the feed costs in hatchery. Earlier studies also informed that availability of red alga, *Pyropia yezoensis* is high in the many countries and they contain the highly concentrated protein. As we know, broodstock diet needs more protein along with other nutrients; the researchers must also put significant efforts on preparing the broodstock diets which contain all the required ingredients (Inomata *et al*., 2016) [72].

**Fish meal replacement**

The preparation of costless diet is a worldwide challenge to aquaculture industry today and therefore, aquaculture nutritionists must focus more research on these areas. The cost of fish meal used as a major protein ingredient in the fish feed is keep increasing in the market today. For instance, the price of the fish meal during the year 2007 was only Rs. 60. and now it reaches Rs.75. Moreover, the obtainability of fish meal in the market reduced due to many reasons that includes, low catch of less valued fish for fish meal preparation and some ethical concerns. As an alternative, the focus today among the fish nutritionists is that expansion of cost-less plant based ingredients, instead using high-cost fish meal (Craft *et al*., 2016) [73]. Therefore, it can be argued that maximising the plant ingredients by replacing the fishmeal is inevitable for the aquaculture sustainability. However, it needs to explain in the research study that replacing plant ingredients with fish meal does not affect the physiology of rearing animals, which is discussed below.

**Strategies proposed to expand the plant based feed ingredients in the fish diet**

It is proven in the study that the supplementation of some potential additives will improve the digestibility and utilization of plant based ingredients in the diet. Fermentation technology got considerable attention in the feed industry in the recent years. The study of Lee *et al*. (2016) [74] reported that supplementation of 20% fermented soybean meal (FSM) in the diet can replace 40% of fish meal in the diet without having any negative impact to rockfish. It was also seen that the plant ingredients based diet improves the intestinal microbiota, immunity and stress in Senegalese sole when supplemented along with either probiotics or immunostimulants (Batista *et al*., 2016) [75]. Lysine is the limiting AA in the many cereals and plant feedstuffs. Therefore, when we expand the more plant ingredients in the diet, it is also necessary to study the optimum dietary lysine requirement for the growth of fish (Michelato *et al*., 2016) [76]. Benedito-Palos *et al*., 2016 [77] supported that fish meal or fish oil can be either partially or fully replaced by the supplementation of butyrate as an additive in the feed as they have positive role to tackle any negative effects to it. It was also showed that supplementation of taurine in the low fish meal diet improved the growth performances of white grouper (Koven *et al*., 2016) [78]. Methionine supplementation is important for plant based feedstuffs. Previous study noticed in the rainbow trout that deficiency or excess of methionine in the diet disturbs the hepatic intermediary metabolism. Therefore, an accurate amount of essential amino acids (EAAs) such as methionine should be present in the diet (Skiba-Cassy *et al*, 2016) [79]. Wendy *et al*., 2010 [80] have found that the supplementation of either probiotic bacteria or commercially produced anti-inflammatory antibodies will improve the utilization as well as health of the digestive tract. It has been found that microalga, *Phaeodactylum tricornutum* in the diet of Atlantic salmon can replace the fishmeal up to 6% in the diet without affecting the nutrient digestibility, growth and feed utilization (Sørensen *et al*., 2016) [81]. Earlier study revealed that it would be even possible even to make the diet deficient in the fish meal since the fishmeal-free diet offered to the tilapia by adopting alternate day feeding strategy showed no negative impact on the animal performances (Borski *et al*., 2011) [82]. The application of exogenous protease in the diet will also be the best strategy when the plant feedstuffs are expanded in the diet as they have less protein digestibility. Gibel carp when fed with 150-175 mg/kg protease in the pelleted low fish meal diet containing 30 g/kg fish meal had significantly improved the animal growth and nutrient utilization; also improved the retention of protein and lipid (Shi *et al*., 2016) [83]. However, enzymes are costly; need to be optimized for cost effective usage in the feed. Food manufacturing industries release huge quantities of wastes containing the great amounts of nitrogen, phosphorus, and some other nutrients. Those can be collected, washed and prepared as a feed (biofloc) through biological reactors for making costless feed. An attempt has been made to test this. It recommended that harvested bioflocs obtained from the biological reactor can be used in the shrimp feeds to replace fishmeal or soybean (Kuhn *et al*., 2016) [84].

Plant ingredients have anti-nutritional factors (ANFs), but that can be removed by proper techniques such as washing, heat, chemical and biological treatments. Increasing plant based ingredients in the diet may lower the bio-availability of trace minerals in the diet due to the presence of ANF such as phytic acid in the plants. In order to solve this, previously inorganic trace minerals used in the feed formulation, but due to their high affinity towards the anti-nutrients in the plant proteins, it becomes a problem. But, it will be solved by supplementing chelated trace minerals as they found to improve the nutrient digestibility and growth. It was found that when Pacific white shrimp fed with chelated trace minerals (Cu, Zn and Mn) in the premix had more efficacy than that of inorganic trace minerals (Cu, Zn and Mn) (Katya *et al*., 2016) [85].

As the plant based ingredients will be expanded in the future, fish has to compel to eat the food, but that is not efficiently possible. Chemo-attractants has role in these concerns as they stimulate the feeding behaviour. It was found that krill meal in the diet improves the palatability in Pacific white shrimp (Derby *et al*., 2016) [86]. Similarly, inosine and inosine monophosphate are the feeding stimulants improve the feeding behaviour when studied in red sea bream (Hossain *et al*., 2016) [87]. In order to ensure the preparation of costless feed with more plant based ingredients and less fish meal content, one must consider the strategies discussed here. This will help feed the farming animals sustainably.

### Fish oil replacement

Fish oil is the common nutrient used in the fish feed as it rich in long-chain polyunsaturated fatty acid (LC-PUFA) which is vital to animals. But due to its less supply, high demand and high cost now it gradually decreasing in the fish feed. The price of fish oil in the market became costly (Rs. 100 per Kg) and less accessible to feed manufacturers as there is a limited supply of marine capture fishery to produce this. By considering the beneficiary state of fish farmers around the world, the research focus established to find out the best feedstuffs to replace the fish oil in the fish diet.

### Approaches established to replace the amount of fish oil used in the fish diet

The attempt has been made to replace the menhaden fish oil with alternative plant lipid sources in the carnivorous marine water fish, Pompano. It found that fully hydrogenated, SFA-rich soybean oil can be replaced in the diet in place of fish oil (Rombenso *et al*., 2016) [88]. Zhang *et al*. (2016) [89] reported that in the bullfrog, fish oil can be solely replaced by soybean oil (SO) or palm oil (PO) for 8 weeks with no negative impact to the growth of the fish. Previous study also suggested that the effect of krill oil is equal to fish oil; hence, fish oil can be replaced by krill oil (Salini *et al*., 2016) [90]. Benedito-Palos *et al*., 2016 [77] supported that fish meal or fish oil can be either partially or fully replaced by the supplementation of butyrate as an additive in the feed as they have positive role to tackle any negative effects to it. The study was also conducted in *Shortfin corvine* to find at what degree soybean oil (SO) can replace the fish oil in the diet. Results found that soybean oil can be replaced up to 50% of fish oil in the diet without any negative impacts to the animals (González-Félix *et al*., 2016) [91].

### Soybean meal replacement

At present, the considerable current research focuses on replacing the fish meal with soybean meal in the fish diet. But, at present, the availability, demand, high costs of soybean meal along with fish meal threatens the aquaculture to manufacture cost efficient diet. So, in the recent years, the replacement of soybean meal also got considerable attention in the aquaculture. Recently, study has been carried out with Lipid-extracted distillers dried grains with solubles (LE-DDGS) in the tilapia diet by replacing soybean meal. It was noticed that with the lysine supplementation LE-DDGS can able to replace as much as 500 g per kg of soybean meal in the diet (Chatvijitkul *et al*., 2016) [92]. In addition, adoption of certain technology include, fermentation technology, exogenous addition of digestive enzymes or addition of deficient nutrients in the other costless ingredients, soybean can be replaced in the fish diet.

### Other strategies developed for the production of aquatic animals in a cost less way

Apart from fish meal, fish oil and soybean meal replacement there are some other strategies have also been applied to the fish farming for the cost less production of fishes to ensure more profitability. One of them is alternate-day feeding strategy. It was observed that when Nile tilapia fed in this situation had same body size equal to the animals reared in the control systems (Bolivar *et al*., 2016) [93]. It is also suggested that dietary inclusion of benthic matter (Wen *et al*., 2016) [94] or macro-algae (Wen *et al*., 2016) [95] can be used as an ingredient in the feed. The identification of novel ingredients or additives can help in the preparation of costless ingredients with locally available feedstuffs (Coffey *et al*., 2016) [16]. It is suggested that biofloc technology can be useful for the reduction of feed usage. Recently, study was also guaranteed with exogenous addition of probiotic bacteria in the biofloc system with a view to further improve the growth and immunity by dominating the specific beneficial strains (Krummenauer *et al*., 2014) [96]. Report says, temperature has significant effect on animal growth and feed utilization. It was found that at higher water temperatures of 37 °C, barramundi, *Lates calcarifer* had increased protein to energy ratio in than that of low temperature of the 30 °C (Glencross *et al*., 2010) [97]. So, the study must also be carried out whenever new additives or ingredients are promised to have effect on animal feeding and growth physiology to find out whether temperature further alter any changes. If observed so, the optimum temperature has to be standardized.

### Immunostimulants in fish feed

The disease invasion is the identified problem for many years in the fish farming. Most of the researchers are working on the identification of potent immunostimulants that can protect the animals from the diseases. The fish stay in the water has wide chances of bacterial, parasites and viral attacks. Addition of immunostimulants to the feed claimed to have immunostimulatory activity in fish. Immunostimulants are any substances that contain immunostimulatory action to the animals. There are different components will come under immunostimulants discussed in this subsection. Bioactive components such as fucoidan are used in the fish diet to elicit the immune responses. Nutraceuticals come under the immunostimulants; this concept was originated many years ago; let feed become medicine and medicine become feed is a principle of this. In fish many studies have proven that nutraceuticals have potent effect in the growth and immunity. Studies also found that administration of rare earth elements in the fish diet improve the growth, survival and immunity of fish (Musthafa *et al*., 2016) [98]. Probiotics also come under the immunostimulants as they demonstrated to stimulate immunity in the animals. It is found that supplementation of probiotics, *Lactobacillus acidophilus* in the diet of snakehead improved the growth performance, nutrient digestibility and the expression of immune regulatory genes (Munir *et al*., 2016) [99]. World is blessed with thousands and millions of medicinal plants; they too claimed to have immumo-modulating effect for the cultured aquatic animals. The nucleotides such as inosine and inosine monophosphate are the functional nutrients have potential effect on the growth, immune response, stress resistance and gut morphology in fish (Hossain *et al*., 2016) [100]. It was found that nucleotides at the rate of 1.0-1.5 g kg- 1 in the diet promoted the growth, immunity and oxidative stress resistance to red sea bream (Hossain *et al*., 2016) [87]. It was also documented that fish fed with Mannan oligosaccharides (prebiotics) in the diet gave better health performance and disease resistance to animals (Torrecillas *et al*., 2014) [101]. The organic acids such as citric acid and their salts are used as an additive in fish feed for the growth and utilization of nutrient in fish (Romano *et al*., 2016) [102]. Biofloc system is famous for its growth and immune enhancing property to the animals. Studies suggesting that it has both growth and immuno-stimulatory activity.

Now replacement of antibiotics became famous, need alternative therapeutic methods. Sudheesh *et al*. (2016) [103] suggested that functional feeds containing immunostimulatory action will be good strategy to overcome the current coldwater disease problems to the salmon. Salmon farming have many disease threats including salmonid rickettsial septicaemia (SRS). Hernández *et al*. (2016) [104] suggested that commercially manufactured phytopharmaceuticals from herbal and macroalgae have potent effective against this disease. Bilen *et al*., 2016[105] carried out the research to find out if the plant extracts can be replaced with cheapest oyster mushroom and nettle methanolic extracts for immune and disease résistance response to rainbow trout and they found positive results. It was found that 2 mg of dietary copper nanoparticles (Cu-NPs) in the diet improved the growth, immunity and anti-oxidant systems than that of copper sulfate ($CuSO_4$) in red sea bream (Basuini *et al*., 2016) [106]. *Mentha piperita* is the essential oil, which is toxic at higher dose and found that which is effective against the parasite attack at the dose of lower than 25 mg $L^{-1}$ s in *Arapaima gigas*. In this regards, the optimum dosage levels needed for the parasite elimination has to be properly addressed (Malheiros *et al*., 2016) [107] for therapeutic use against parasites.

### Quality aspects of feed

The preparation of quality feed is a matter for every aquafeed formulators. However, this is really a challenge, because ingredients or feed contains considerable amount of lipids and lipid soluble nutrients which are very much susceptibility to oxidation and easy to spoiled by bacteria and other fungal attacks (Qiu *et al*., 2016) [108]. Despite synthetic antioxidants was used in higher dosages in feeds, it was criticized due to residue and other concerns. The availability of most ingredients is unstable and depends on seasons; hence, keeping them without any quality related problems is very crucial in the feed mills. Mycotoxin (T-2 toxin) is caused by fungus *Fusarium sp* is the major contaminant in the animal feeds. When shrimp fed with the T-2 toxin contaminated feed it affects the hepatopancreas, hemolymph and immune system, which also further leads to decreased growth and survival. Some preservatives such as organic acid (sodium citrate) are often added in the diet which improves the shelf life and improves the oxidative stability of feed (Romano *et al*., 2016) [102]. However, there are relatively very few studies in feed nutrition focussing on Mycotoxin alleviation from either feed ingredients or feed as compare to other feed oriented studies. Thus, the critical need of research is required in this field.

### Environmental nutrition

There are various environmental concerns pertaining to the aquaculture industry are focussed here, which must be addressed in the effective ways. For environmental friendly aquaculture, the following considerations can be made to improve the feeding and so prevent the water pollution caused by uneaten feeds. The wastage of fish feeds or ejected faeces contains the great sources of nitrogen and they are toxic to fish. Adoption of some techniques such as periphytons or biofloc along with less protein feeding it is possible to ensure maximum feeding capacity of animals and this can also eliminate raising the nitrogen content of the feed (Crab *et al*., 2012) [109]. The periphytons or biofloc contains the beneficial bacteria which can utilize the available nitrogen as a substrate for their growth and metabolism. According to Ekasari *et al*. (2014) [110], biofloc system will ensure the reduction of water quality and pollution problems in the aquaculture. This is the area of research may solve some concerns related to environmental concerns to the aquaculture. Phosphate is a mineral required for animal growth and physiology. It also considered as a polluting nutrients as the utilization of phosphorous vary among the animals, depends on the form. Hence, increase the utilization of phosphate in the feed will lessen the environmental problem as it pollutes the water by high eutrophication (Halver and Hardy, 2002) [1]. It was found that acid hydrolysed phosphorous prepared from herring bone by-products when supplemented in the feed of Atlantic salmon gave positive impacts on growth, mineralization and skeletal development than that of commercially used sodium phosphide (NaP-salts) (Ytteborg *et al*., 2016) [111]. The ornamental fish diets rely on the incorporation of pigments such as carotenoids to improve the coloration. Due to many reasons, the synthetic pigments sources added in the feed s discouraged (Daniel, 2016) [112]. Therefore, aquaculture has to give much importance to organic farming as the synthetic addition of any additives will be discouraged in the industry. In this connection, natural feed ingredients contains more pigments in it must be explored for feed usages.

Salinity plays an important role in the growth, osmoregulation and energy turnover in the aquatic animals. Pompano is a euryhaline fish mostly reared in the higher salinities till 32 ppt as it is marine water species, and it would not be cost effective if reared in the fresh water. But the finding from the study shows that it had higher growth in the low (3-6 ppt) salinities (Anni *et al*., 2016) [113]. The adjustments of body temperature to water temperature animals spend energy that can be saved if animal's best growth for water temperature is properly optimized. Digestive tract of animals have huge amounts of bacteria, but if affects by environmental conditions. However, there is very less knowledge on how the salinity alters the change of microbiota in the intestine of fish.

Nile tilapia and pacific white shrimp are euryhaline species; they can tolerate any salinity. But to see whether changing the salinity play a role in the changing in the intestinal microbiota, there was no information related to it. It was found that increase or decrease in the salinity play a role in the alteration of bacteria resides in the intestine (Zhang *et al*., 2016) [114]. Research has to establish in this area. The intensive practice raised in the aquaculture today has elevated the question if high stocking density threatens the environmental issues to the aquaculture. Some studies suggest that high stocking density affects the growth and survival of animals. However, some study declares that they have no difference than that of normal stocking density systems (He *et al*., 2016) [115]. These problems must be solved by increasing the number of supporting works in these areas. The quality of fish i.e. organoleptic properties of the animals must be improved. A barramundi was fed with sea lettuce, *Ulva ohnoi* for a period of only 21 days (Finishing diet) and found that organoleptic properties such as taste, flavour and aroma in the animals were improved. This will facilitate the attraction of animals by the consumers and also stimulate the export of value of animals. This will also support feed formulators to avoid adding some synthetic additives in the diet and prevents environmental issues (Jones *et al*., 2016) [116].

**What is expected for focus in the future?**

- ✓ Development of larval feed.
- ✓ Replacement to fish meal and fish oils.
- ✓ Expansion of plant feedstuffs.
- ✓ Different less expensive protein feedstuffs has to be explored for feed inclusion.
- ✓ To improve the performances of fish digestion and utilization of ingredients, the considerable research must focus on the selection and exploration of some novel feed additives.
- ✓ The study must focus on following aspects when different alternative dietary materials are given to fish: In gut what changes takes place? Is it advantage to fish? These should be properly assessed.
- ✓ Influence on animals growth and immune response through the different ingredients used in the feed must be documented.
- ✓ There was a belief that animal feeds do not pose any problems to the consumers. But, some case has been evidenced which confirms that certain diseases are coming to the fish eaters via animal feeds. This is true as feed formulators occasionally add some adulterants to improve the nitrogen or other nutrient contents of the feed. Therefore, technology must be developed to find out the presence of adulterants and contaminants in the fish feeds and that practice must become compulsory during the quality evaluation of feeds.
- ✓ **Nutrigenomics:** It is the study of gene expression with response to dietary nutrients. This approach is used to understand the basics of metabolic changes in animals with response to feeding different dietary nutrients. Since the cell growth is directly related to the animal's metabolic alterations when fed with different nutrients, the results from these kinds of studies will certainly help in the understanding of animal's response to different feed ingredients when fed. This is an interesting area, must be critically explored. (Overturf *et al*., 2016) [117].

**Concluding remarks**

The feed industry is growing, but aquafeed industry is still to develop gigantically to meet the human food demands. The number of feed mills and the production capacity has to be extended to produce more feeds; so that in the future the fish as cheapest food can be sustainably reached to the global population as it is expected to reach 9 billion in the year 2050. Currently, the tools used for feed preparation is less rely on the advance technology such as in vitro fermentation, near-infrared spectroscopy (NIR) and other molecular techniques such as bioinformatics and nutrigenomics. These tools must be intervened and applied for the optimization of nutritional value of feed ingredients and for the feed formulation steps. In the overall conclusion, it is advised to say that aquaculture research commit to focus more on nutrition and feed oriented aspects covered in the review.

**Conflict of interest statement**

Author declares that there is no conflict of interest in the manuscript.

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