FORMULATION OF FISH FEED USING MANGROVE LEAVES *(AVICENNIA OFFICINALIS)*

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ABSTRACT : Aquaculture and fisheries are important sectors of food production, providing nutritional security to the food basket, contributing to the agricultural exports and engaging about fourteen million people in different activities. Commercial aquaculture is growing thereby increasing the demand of feeds. Traditionally these feeds are based on animal protein however due to the cost and availability consideration it is inevitable that more plant proteins supplements will be utilized in the feeds in the future. The increased acceptability and utilization by the feed industry will be depending on reliable nutritional research. The feed ingredients tend to be mostly by-products of processing or milling industries, but also consist of natural products. In formulation of diets, these ingredients are included and substitutions made within mixtures in accordance with market price, local availability and composition. Taking into account these considerations, present formulation is undertaken by using plant origin source as mangrove leaves (*Avicennia officinalis*). Mangrove leaves contain biological active antiviral, antibacterial and antifungal compounds. Knowledge of the biological activities and chemical constituents of mangrove is desirable, not only for the discovery of new therapeutic agents, but also in disclosing new sources of already known biologically active compounds. For the said reason Mangrove leaves are used as base in feed formulation. Other ingredients used are of high nutritive value. The feed is well accepted and easily consumed by herbivorous fishes.

Key words: Feed formulation, mangrove leaves, and biological active, antiviral

INTRODUCTION:

For a long period of time in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (Bandaranayake, 2002, Kokpal, 1990 and Walsh,1977). Even today people who live near to the forests use plant products to cure chronic diseases. Tropical and sub-tropical areas of the world are bestowed with abundant flora and herbs which have untapped properties, such as antimicrobial, antiviral and antifungal. According to the World Health Organization, plants are a source of compounds that have the ability to combat disease, antimicrobial, antiviral and antifungal activities (Watson, 1929). In addition, medicinal plants have been used for centuries as remedies for human ailments and diseases because they contain components of therapeutic value (FAO, 1982). Also they are less toxic to humans and environmentally friendly due to less pollutants produced in production and have minimal health hazards (Bandaranayake,1998). A large amount of revenue of the world has to pay for the health care. Day by day new dreaded diseases are arising. The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world and infectious diseases are the second most serious cause of death worldwide both to man and animals (Bandaranayake, 1995). Therefore, new drugs have to be found, in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties.

Concerning the above facts, it is worthwhile to screen plant species which have the above properties to synthesize new drugs (Bandaranayake,2002). Mangrove forests are productive ecosystems and support a high abundance and diverse variety of wild. The importance of mangrove leaf litter in the maintenance of detrital-based food webs in the coastal environment and their significance for coastal fisheries has been indicated for some time (Golley *et al.,* 1962; Odum & Heald, 1975; Ong *et al.,* 1984;)There is a rich species composition and 4000 ha of mangroves are present in Sri Lanka (Premnathan *et al*. (1992) and extracts from different mangrove plants are reported to possess diverse medicinal properties(Premnathan *et al*.1996). Mangrove have been a source of several bioactive compound. Mangrove plants have been used in folkfore medicine and extracts from mangrove leaves have proven activity against human, animal and plant pathogens.Phytochemical screening of mature leaf revealed that secondary metabolites like alkaloids, phenolics, steroids and terpenoids have been characterized from mangrove leaves and have toxicological, pharmacological and ecological importance. *Avicennia officinalis* showed significant analgesic activity (Miki *et al*., 1994, Ishibashi *et al*., 1993). A number of mangroves and associates contain substances which show biological activities such as leaves of *A. officinalis* are used to treat rheumatism, neuralgia and poison arrow wounds. The antibacterial activity of the leaves and bark of mangrove plants, *A. officinalis* was evaluated against antibiotic resistant pathogenic bacteria, *Staphylococcus aureus* and Proteus sp. Most of the plant extracts showed promising antibacterial activity against both bacterial species. However, higher antibacterial activity was observed for *Staphylococcus aureus* than Proteus sp. The highest antibacterial activity was shown by ethyl acetate of mature leaf extracts of *A. officinalis* for *Staphylococcus aureus*. All fresh plant materials did also show more antibacterial activity against both bacterial strains than did dried plant extracts. Medicinal uses of *A. officinalis* include using the powdered leaves to relieve constipation and a tea brewed from the leaves to treat fish poisoning.

MATERIALS AND METHODS :

Basically, the concept of feed formulation is to use available ingredients in the most economical way to provide the essential nutrient content and balance of the final diet. Different proportions of less expensive ingredients can often be combined to achieve the nutrient balance of more expensive ones. However, it is also necessary to consider factors such as the quality, palatability and functional properties of ingredients as well as the possible content of anti-nutritional components that are known to affect the growth and health of fish. Protein is usually the first nutrient considered, with the level of energy in the diet being adjusted to provide the optimum ratio. The protein has to be balanced for essential amino acids. It is the main constituent of the fish body. Plant protein is generally cheaper than animal protein supplements. Hence in the present study the mangrove leaves were used as source of protein to formulate the feed.

Formulation of fish feed: The fresh green mangrove leaves were collected, brought to the laboratory, washed and cleaned using distilled water. The paste of the same was prepared using mortar and pastel. Ingredients such as milk powder (60 gm), corn flour (20 gm), and eggs (70 gm) were added and mixed well. Agar powders (4 gm) were added as binding agent; turmeric, pepper and cumin powder (each of 0.5gm) and garlic paste (1 gm) were added as antibiotics. Cod liver oil (3.5 ml) and vitamin mixture of B and E (each of 1gm) were added. Ingredients in semisolid form were kept under refrigeration for 12 hrs. Then they were squeezed over polythene sheet and dried at room temperature for 48 hrs. The dried nodules were crushed into small pellets. Pellets were sun dried to avoid fungal infection, weighted, stored in the bottles and were used as and when required.

The nutritional importance of ingredients used:

Mangrove leaves : Mangrove leaves contain biological active antiviral, antibacterial and antifungal compounds. It contains secondary metabolites like alkloids, phenolics,steroids and terpenoids.

Milk powder: It is add in feed formulation for its nutritional properties. It contains 20 standard amino acids. It is rich in soluble vitamins and minerals. According to USAID the typical average nutrient in the unreconstitued milk are 36% proteins, 52% carbohydrates, particularly lactose and calcium 1.3%. It is fortified with vitamin A and D (USAID -Jan.2004). Nestle Everyday milk powder is used in present feed formulation.

Nutritional value of milk powder / 100 gms. Protein: 20.5 gm, Carbohydrates: 52.7gm, Fats: 19gm, Saturated Fatty Acids: 10.9 gms, Cholesterol: 0.05 gm, Mono Unsaturated Fatty Acid: 4.21gm, Poly Unsaturated Fatty Acid: 0.41.Partly skimmed milk along with sucrose is the ingredient present in used milk powder. (USAID 2004).

Egg: Egg has two main components, yolk and egg white. Egg white is taken as ingredient during feed formulation. Egg white is common name for clear liquid called as albumin or glair or glaire within an egg. It is the cytoplasm of the cell. It contains 15% of protein dissolved in water. It contains about 40 different types of proteins. It has high nutritive value. Fat content is negligible. The proteins in egg white are, ovalbumin: 64%, ovatranseferin: 12%, ovomucoid: 11%, ovamucin: 1.5%, globulin: 8%, lysozyme: 3.5%.
(USAID 2004).

Corn flour: Corn flour was used as filler and binder in feed formulation. It contains proteins: 3 gms, carbohydrates: 23gms and fat: 1gm. The major ingredients were maize and starch. Starch acts as additive in food processing. It has 110 calories per gm. (USDA 2008).

Cod liver oil: Cod liver oil is derived from the liver of cod fish *Gadus callarias*. It has high levels of omega-3 polyunsaturated fatty acid chain of EPA (Eicosapentaenoic acid) and DHA (docosahexaenoic acid). It is a good source of the vitamins A and D. It contains small amount of fish protein which gives fishy smell to it (Aviram M, Brox J. Nordoy A, 1986).Cod liver oil along with vitamin E prevents the body from increasing oxidant stress. It reduces the tendency of blood forming clot and helps to reduce generation of free radicals in body.

Vitamin mixture: Vitamin B and E are used as vitamin mixture in equal proportions. Both vitamins are in the form of capsules. Each capsule of vitamin B complex is composed of Thiamine mononit- rite, vitamin B2, B6, B12, nicotinamide, folic acid, biotin and titanium dioxide. It promotes activities of enzymes and plays an important role in cell growth and metabolism (Vera Reader, 1930) and is an essential nutrient for the growth, development and other bodily functions (Guyton JR., Boys HF., 2007).

Vitamin E is the soft gelatin capsule containing tocopherol acetate of about 400 mg. It is a powerful antioxidant and protects the body against the free radicals which are potentially damaging byproducts of energy metabolism.

Agar powder: Agar powder is used as binding agent that binds loose mixture together. It helps to form a unique mass of the feed. It is a polymer made up of subunits of sugar galactose. It is acts as an emulsifying and suspending agent in food products.

Turmeric powder: Scientific name of turmeric powder is *Curcuma longa* and belongs to the family: Zingiberaceae It is selected for its medicinal properties such as: antibacterial, anti-inflammatory and hepatoprotective. It functions as antioxidant and adjuctant in feed preparation and is used to protect the food from sunlight (Shrinivasan M and Satya Narayan, 1987). Turmeric adds warm, mild aroma and distinctive yellow colour to the feed. It is hypolipidemic (Khanna K. M., Sarine J. P., Singh S, 1904).

Nutritional value of turmeric /100 gms. Moisture: 5.8%, Protein: 8.6%, Fat: 8.9%, Carbohydrates: 63.0%, Fiber: 6.9%, Total ash: 6.9%, Calcium: 6.2% and, Iron: 0.05%.The caloric value is 390 calories per 100 gm. It also contains 5% essential oil, and 3% Curcumin which is a polyphenol (Sharma S. Agarwal S., Kulkarni SK. 2006 May).

Garlic paste: Garlic has been added to feed formulation for its antibiotic and antibacterial properties (American Chemical society, 2002).

Nutritional value of garlic / 100 g: Moisture: 62.8 %, Protein: 6.3 %, Carbohydrates: 29 % Fiber: 0.8, Total fat: 0.1%, Total ash: 1.0 %, Calcium: 0.03%, Phosphorus: 0.31% Iron: 0.001%, Vitamin C: 13 mg and Nicotinic acid: 0.4 mg. The caloric value is 142 per100 gm.

Pepper powder: The botanical name of pepper is *Pepper nigrum* and belongs to the family Pipperaceae. It has long

been recognized as a stimulant for appetite. In India it is used in number of health problems (H. J. D. Dorman and S. G. Deans 2000). Pepper has two main components volatile oil & pungent component commonly known as pipperine. A pepper contains 0.6 to 2.6 % essential oil vitamins A, B and C.

Cumin powder: The botanical name is *Cuminum cyminum*. It stimulates the secretion of pancreatic enzymes, important in nutrient digestion and assimilation.

The nutritional value of cumin / 100 gms. Carbohydrates: 44.24 gm, Proteins: 17.81 gm, Fat: 1.535, Fiber: 10.5 gm, Iron: 66.36mg, Sodium: 168 mg, Zinc: 4.8 mg, Calcium: 931 mg, Vitamins such as: A: 64 mg, Thiamine: 0.628 mg, Riboflavin: 0.327 mg, Niacin: 4.579 mg, Folate: 10 mg, vitamin C: 7.7 mg, E: 3.38 mg (USDA-2008).

RESULTS AND DISCUSSION

Bacterial diseases are responsible for heavy mortality in wild and cultured fish. The problems in the farms are usually tackled by preventing disease outbreaks or by treating the actual disease with drugs or chemicals. The use of antimicrobial agent has increased significantly in aquaculture practices (Alderman and Michel, 1992). Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture. Decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives (Smith *et al*., 1994).The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases, as antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, screening of antibacterial activity of medicinal plants is very important since vast number of medicinal plants have been used for centuries as remedies for fish diseases.

Mangroves and mangrove associates are widely used throughout the world. Mangroves have been a source on several bioactive compounds. Scanty literature is available on the antibacterial activity of mangroves. However, studies of other biological activities in general are available. The study of Premnathan *et al*. (1992, 1996) revealed that the mangroves were found highly effective for antiviral activity as compared to seaweeds and sea grasses. Kokpal *et al*. (1990) had also reported the bioactive compounds from mangrove plants. Some mangrove plants had shown insecticidal activity (Miki *et al*., 1994, Ishibashi *et al*., 1993). They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins (Bandaranayake, 1995). Antimicrobial activity of plant constituents such as phenol, quinines, flavones, flavornoids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors (Edeoga *et al.*2005).

As there is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases (Rojas *et al*., 2003) hence in the present study mangrove leaves were used in tune with the fish's needs for the maintenance of vital physiological functions such as growth, reproduction and health. The feed was well accepted and easily consumed by the fishes.

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Fresh Mangrove leaves

Formulated feed