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USE OF IMMUNOSTIMULANTS FOR FISH HEALTH MANAGEMENT IN MYMENSINGH DISTRICT OF BANGLADESH

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ABSTRACT

Immunostimulants have been used as a prospective substance against several disease events in aquaculture, although subsequent impact is not clearly explored yet. The present study was conducted to investigate the use of immunostimulants for fish health management in Mymensingh district of Bangladesh. Questionnaire survey was performed with a total of 40 fish farmers and 20 chemical and drug retailers. Altogether, 41 immunostimulants under 7 different categories including glucans and polysaccharides, vitamins, minerals, vitamin-mineral premixes, enzymes, nucleic acids and plant extracts were reported. ACI, Eon, Acme, Square, Renata and SK+F were noticed as the prominent sources among 24 listed companies in this study. The most frequently used immunostimulants were recorded as Megavit-aqua (88.75%), Multivitamin (87.5%), Aqua-boost (87.25%), Eskalina (83.25%) and Eon fish grower (82.5%). Farmers in this study mentioned about improved health condition with lower average disease incidence (29.3%) and reduced mortality (65.35%) in fish after application of immunostimulant. The findings of this study will be useful to perform further research regarding the efficacy and impacts of a wide range of immunostimulants used in aquaculture of Bangladesh.

Keywords: Immunostimulants, Aquaculture, Fish health management

INTRODUCTION

Aquaculture being one of the fast-growing food sectors in the world constantly requires new technologies. Technical innovations have become a key to healthy and productive farmed aquaculture candidates (Mishra et al., 2020) and farming intensity with improved husbandry are playing essential role to aim the quality production. But several disease outbreaks have been always one of the primary limiting factors for

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this industry. Immunostimulants have been used in fish and shrimp farming recently to overcome this challenge whereas antibiotics and chemotherapeutics are mostly used in fish farms and hatcheries against diseases (Jadhav et al., 2006; Suheyla et al., 2003).

Though the preventive measures have come out as partially successful actions for the last 20 years, huge economic loss due to disease is still reporting every year (Harikrishnan et al., 2011*)*. Modern science is working on this issue to lower the economic loss and to ensure the better growth of farmed aquaculture species. Immunostimulants are such a discovery of this era as 'bio-friendly agents' which may be incorporated as biological factors, probiotics and vitamins into the culture environment. It is used to control and kill the pathogenic bacteria and to promote growth of the farmed organisms. Immunostimulants are non-pathogenic and non-toxic, and have been reported with no undesirable side effects when administered to aquatic animals (Wang et al., 2017).

Immunostimulants are mainly used as prophylactic interventions but are not recommended if disease is already present in the host. These agents may have modulatory effects on immunocompetence of fish and crustaceans. Conversely, the application of immunostimulants may compensate for the limitations of chemotherapeutants and vaccines. It may also increase the potency of vaccination when combined with inactivated and some other vaccines. Diverse commercial immunostimulants are available these days in aquaculture. Good efficacy, wide spectrum of activity, no residues and eco-friendly nature of this agent can enhance the non-specific immune system and a very few cases of specific immunity (with vaccines). However, the effects of each immunostimulant are varied depending on its source, dose, route of administration, length of exposure, and the species to which it is administered. Sometimes, overdoses of immunostimulants may induce immunosuppression as well (Amar and Amar, 2015; Dhama et al., 2015; Kunttu et al.,2009).

Various microbial components and plant products such as polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, muramyl dipeptide and yeast derivatives have been used as immunostimulants in aquaculture. So far, glucans as the polymer of glucose appear to be most promising immunostimulant in fish and shrimp through oral administration (Dalmo and Bogwald, 2008; Mastan, 2015). No specific research on the use of immunostimulants in aquaculture have been carried out yet in Bangladesh. The present study was therefore, directed to investigate the use of immunostimulants in fish health management in Mymensingh district of Bangladesh.

MATERIALS AND METHODS

This study was conducted in four upazilas of Mymensingh district namely Mymensingh Sadar, Trishal, Bhaluka and Muktagachha. The primary data was

collected from target groups of fish farmers and veterinary drug shops through pre-tested questionnaire. A total of 40 fish farmers (10 farmers from each upazila) and 20 drug retailers (Mymensingh Sadar-4, Trishal-6, Bhaluka-6 and Muktagachha upazilla-4) were interviewed through structured questionnaire about the use of immunostimulants, their types, active ingredients, sources, most commonly used immunostimulants and their effects on fish health and disease. All the collected data were then integrated and processed in Microsoft Excel 2010 for further analysis.

RESULTS AND DISCUSSION

Immunostimulants used in fish health management

Both monoculture and polyculture farming at grow-out stage fish were considered in this study where farm areas were ranged between 5-10 acres. Pangus (*Pangasius hypophthalmus*) was found as the main monoculture species, while polyculture fish species were Tilapia (*Oreochromis niloticus*), Rui (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*), Gulsha *(Mystus cavasius),* Shing (*Heteropneustes fossilis*), Silver carp (*Hypophthalmichthys molitrix*), Carpio (*Cyprinus carpio*) and Pabda (*Ompok bimaculatus*).

A total of 41 different types of immunostimulants were recorded in this study through 24 distinct companies in the market. Among the companies, ACI, Eon, Acme, Square, Renata and SK+F were mentioned as the prominent ones by the farmers. Depending on the active ingredients and contents, listed immunostimulants were grouped under 7 major categories namely glucans and polysaccharides, vitamins, minerals, vitamin-mineral premixes, enzymes, nucleic acids and plant extracts.

Glucans and polysaccharides

Three glucans including Aqua-boost, Renuzyme and Enzyme vet were recorded from the drug shops. Major constituents were found as β-glucan and β -xylanase. Enzyme vet was the only one in this category which is generally used in aquaculture as off labeled immunostimulants since it is actually prepared for poultry but were seen to use by fish farmers (Table 1). According to the correspondence, all the glucans and polysaccharide based immunostimulants were usually incorporated in the aqua feeds for enhancing immunity of fish. Price was found to vary between 162 and 245 BDT per kg according to the packaging details.

Table 1. Glucans and polysaccharides found in this study

Trade name	Active ingredients	Dose/kg feed (g)	Company
Aqua boost	β-glucan, hemicellulose, mineral, amylase, amino acid, pectinase	0.5	Elanco
Renuzyme	β-glucan, amylase, lipase, *Bacillus subtilis*, protein fat	0.5	Star Agro Product
Enzyme vet	β-glucan, hemicellulose, minerals, amylase, amino acid, pectinase	0.5	Biocare

Vitamins

Eighteen vitamins with major active ingredients as vitamin C were found in the animal drug shops (Table 2). Some of the vitamins were administered through feed and some in water according to the instruction given in the level of the product. Farmers also mentioned that they sometimes follow the dose instruction from the pharmaceutical representatives. The price of the vitamins was found as minimum 60 BDT/per kg for BC mix (B+C) and maximum 394 BDT/kg for liverzyme vet (Table 2). Farmers applied vitamins to increase growth and to reduce the vitamins deficiency in fish. Farmers have experienced better fish health after applying vitamins in fish.

Table. 2. Vitamins found in the drug shops in the study areas

Vitamins applied through feed			
Trade name	Active ingredients	Dose/Kg feed (g)	Company
Bio-C	Vitamin C	2.0	Aquatech
Vital-C	Vitamin C premix	3.0	Navana
Nutr-C	Water soluble vitamin C premix	2.0	Nutriforti Ltd.
Cevit aqua	Vitamin C	1.0	Square
BC mix B+C	Vitamin (B & C)	2.5	Opsonin
Revit -C	Vitamin C	0.7	Opsonin Pharma
Eskavit-C	Vitamin C	2.0	SK+F
Safe gut	Vitamins & enzyme	2.0	SK+F
Liverzyme vet	Vitamin B1, B12	1.0	Golden Agrovet
Panvit-aqua	Liquid multivitamin- Vitamin (A, C, B1, B2)	1.0	Square

Vitamins applied in water			
Trade name	Active ingredients	Dose/L water (g)	Company
Vitax- BC	Vitamin (C, B2, B5, B6, B12), Nicotinamide, Folic acid	2.0	Eon
C-plus premix	Vitamin C	1.3	Agrovet
Energy plus	Vitamin C, Glucose	2.0	ACI
Biomix-c	Vitamin C	0.1	Techno
Oralite	Vitamin A	1.2	Opsonin
Rena-C	Vitamin C	0.2	Renata
Glucolite	Vitamin A	1.2	Acme

Minerals

Two minerals (DCP-Plus and DCP-Gold, off labeled) were detected as the most commonly used immunostimulants to increase the growth and protection against overall diseases in farmed fish as opined by the farmers. Calcium, phosphate, magnesium sulphate, trace elements and sodium chloride were the major active ingredients of these mineral products (Table 3). Price ranged from 52 to 70 BDT for each kilogram.

Nucleic acids

According to the retailers, nucleic acids were mentioned as a very important immunostimulants for fish because of various physiological and biochemicals functions. The listed nucleic acids (Han milk for fish and Krill meal) are described in Table 3 with active constituents as nucleic acid, vitamin, fat, asthaxanthin and omega enriched vitamins, fat and mineral components. Price varried from 135 to 390 BDT/kilogram. Nucleic acids were applied by the farmers in general during fish transportation to avoid stress in fish.

Enzymes

Enzymes (Acmezyme, Autozyme, Rena-phytase, Saltose plus and Safe gut) were mainly applied with feed in fish by the farmers as a growth and immunity stimulator containing elements like Multi enzyme premix, enzymes, vitamins and probiotics (Table 3). Some enzyme products were found as quite expensive immunostimulants with maximum price of 1125 BDT/kilogram (Saltose plus) while few with reasonable price like Rena-phytase for 150 BDT/Kg. Krill meal, Acmezyme, Autozyme and Saltose plus were used as off-label immunostimulants.

Plant extracts

Only one type of immunostimulants from plant extracts was used by the fish farmers incorporated into the feed. The trade name was reported as Eskalina which is actually found as a rich source of safe and inexpensive chemical compounds as per the level of the product. The manufacturing company was SK-F and the major constituent of

this immunostimulant was observed as organic spirulina (*Arthrospira platensis*). Farmer were usually getting this product with a price of 300 Tk/kg and were applying at a dose of 5g/kg in fish feed.

Table 3. Minerals, Nucleic acids and Enzymes found in the study

Categories	Trade name	Active ingredients	Dose/Kg feed (g)	Company
Minerals	DCP-Plus	Mineral salts: Calcium, phosphate, magnesium sulphate, trace element	-	Opsonin
	DCP-Gold	Calcium, phosphate, magnesium sulphate, mineral (Fe, I, Cu, Zn, Co, Mn) sodium chloride	-	Confidence pharma Ltd.
Nucleic acids	Han milk for fish	Nucleic acid, vitamin and fat	-	Hanvet
	Krill meal	Asthaxanthin and omega enriched (protein, fat, mineral)	2.0	ACI
Enzymes	Acmezyme	Multi enzyme premix	0.5	ACME
	Autozyme	Multi enzyme premix	0.5	ACME
	Rena-phytase	Lysine, Isolysine, arginine, histidine, mineral, protein	1.5	Renata
	Saltose plus	Enzyme & probiotics, Beta-xylanase, cell wall lyase, Bacillus group, enterococcus	0.5	Opsonin
	Safe gut	Enzyme & Vitamins	2	SK+F

Vitamin-mineral premixes

Eon fish grower, Revit ES, Orgal-p-plus, Vitatech, Provit super, Gamma organ, Megavit aqua, Chemovit DB and Eskalina were found as the commonly used Vitamin-mineral premixes. Vitamin A, Vitamin B, Vitamin C, Calcium, phosphate, Antioxidant, selenium, mineral, Vitamin-mineral premix, Amino acid and magnesium were found as the active ingredients. Revit ES, Orgal-p-plus, Chemovit DB were used as off-label immunostimulants among all the listed products. Most of the vitamin- mineral premixes products were applied in with feed following different dose (Table 4) excluding nutrigel (from SK+F) which was usually applied with water at (10 ml/Kilogram). Market price was widely varied from 120 BDT to 550 BDT for each kilogram. Revit ES, Orgal-p-plus, Chemovit DB were used as off-label immunostimulants.

Table 4. Vitamin -Mineral Premixes from this study

Trade name	Active ingredients	Dose/Kg feed (g)	Company
Eon fish grower	Vitamin mineral premix: Vitamin (E, D, E, K) Mineral, Calcium, Antioxidant	3.0	EON
Revit ES	Vitamin & selenium	1.0	Opsonin
Orgal-p-plus	Vitamin & mineral	2.0	International Biologicals
Vitatech	Vitamin mineral premix: (vitamin A, B1, B2, B6, D, E) Niacin, calcium	5.0	Aquatech
Revit ES	Vitamin & selenium	1.0	Opsonin
Orgal-p-plus	Vitamin & mineral	2.0	International Biologicals
Megavit aqua	Vitamin, mineral & amino acid (vitamin B1, B2, B6, B12, D, K, Biotin	1.0	Elanco
Chemovit DB	Vitamin & mineral (Vitamin A, B complex, D, E) Zink, copper, iron, calcium carbonate	3.0	Chemist Laboratory
Eskalina	Vitamin (B1, B2, B6) mineral	10.0	SK+F

Frequent use of immunostimulants by the farmers

More than 80% of the most frequently used immunostimulants were recorded as Megavit-aqua (88.75%), Multivitamin (87.5%), Aqua-boost (87.25%), Eskalina (83.25%) and Eon fish grower (82.5%). Even the lowest (43.75%) frequently used immunostimulant (Enzyme vet) was about half of the stated immunostimulant application (Figure 1). Farmer from the study areas stated about the above-mentioned immunostimulants in the farms for different health management purposes like to prevent skin and fin erosion of fish, to increase the survival rate and to reduce the mortality rate of fish to enhance the immunity of fish, to supply sufficient nutrients (vitamin and minerals) to avoid deficiency, and to control stress and disease in fish.

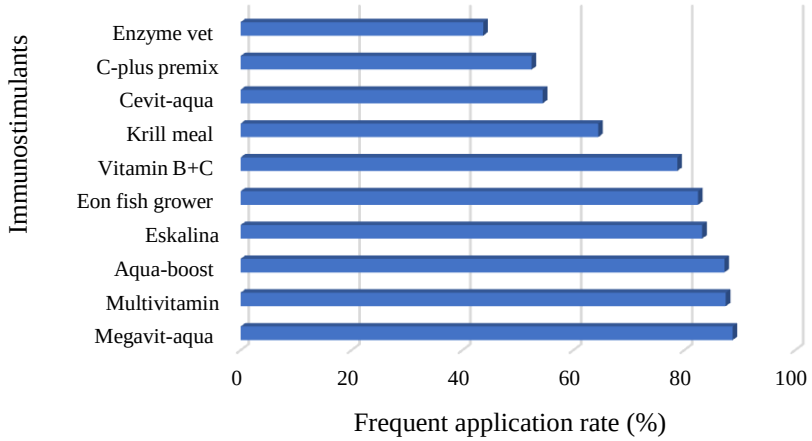

Figure 1. Most frequently used immunostimulants (%) by the farmers

Effect of immunostimulants on fish health and diseases

Majority of the interviewed farmers mentioned about improved health condition with lower average disease incidence (29.3%) in the farmed fish after using different immunostimulants. Before the use of immunostimulant, the highest disease incidence was found in Muktagacga (71.6%) and in Mymensingh Sadar (71.5%). However, after the use of immunostimulants, both of the areas had the lower disease events (28.5%) (Figure 2). Average 65% farmers mentioned that the mortality rate of their farmed fish reduced after using immunostimulants. Among four study areas, highest reduced mortality was recorded in Trishal (72.2%), while the lowest was in Mymensingh Sadar (58.6%) (Figure 3). Reduced mortality in fish experienced by the farmers might be due to the inefficiencies or inappropriate usage of a few immunostimulants from different companies.

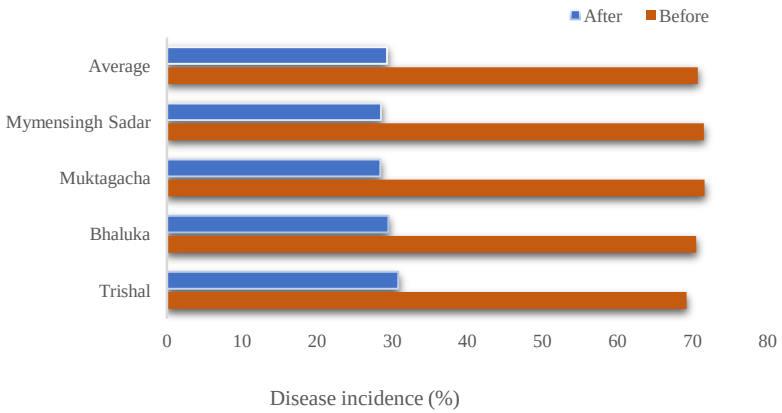

Figure 2. Disease incidence (%) changes upon the use of immunostimulants

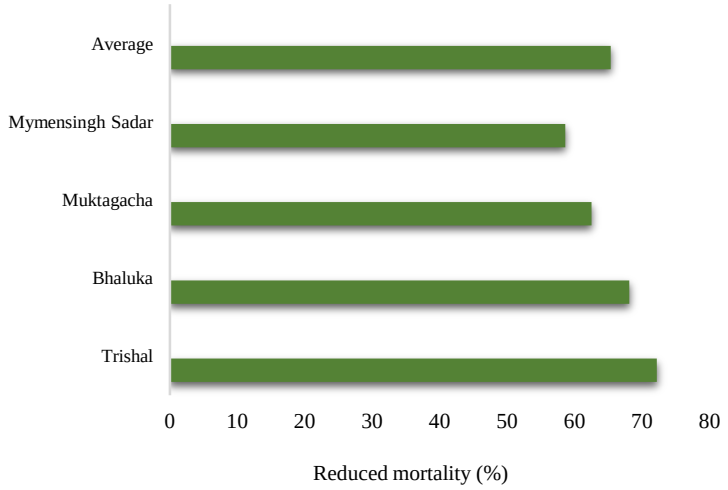

Figure 3. Reduced mortality (%) due to immunostimulant application

To achieve successful production, chemicals and drugs are commonly used in aquaculture of Bangladesh. It is a very common practice by the farmers with a view to maintaining good health, reduced pathogen susceptibility and stress. However, it may impose multiple adverse impacts on the organisms and the environment onwards which is urging us to think about the emerging impact issues and respective solutions. Immunostimulants and nucleotides have such a potential to reduce susceptibility to various stressors and diseases as well as to enhance the overall health of fish (Ringo et al., 2012). Different types of immunostimulants are used in the fish farming of Bangladesh but not well documented. All the above-mentioned immunostimulants are classified based on their major constituents, although immunostimulants can also be classified based on the origin and mode of action (Labh and Shakya, 2014) under the groups like synthetic chemical agents, bacterial products, polysaccharides (complex carbohydrates), animal or plant extracts, vaccines (antigens and adjuvant), immunoenhancing drugs, nutritional factors and cytokines (Barman et al., 2013; Vijayan et al., 2017, Shahbazi and Bolhassani, 2016). In a recent review article about the application of immunostimulants in aquaculture, the author also mentioned about the major immunostimulant classes as polysaccharides, nutrients, oligosaccharides, herbs, antibacterial peptides and microorganisms (Wang et al., 2017).

In this study, it was found that several glucan products were found commercially available and were used in fish farms. It has been found that, a majority of the immunostimulants have come from bacterial derivatives including the main constituents as β-glucan which is a promising stimulators of non-specific defense mechanism in fish. β-glucan is found as a homopolysaccharide of glucose molecule

linked by the glycoside bond. It forms the major constituents of cell wall of some plants, fungi, bacteria, mushroom, yeast, and seaweeds. Research has also been reported that β-glucan dosages, quality, route, and time of administration and duration of treatments can play a significant role in enhancing the growth, survival, and immunity (Meena et al., 2013). Enhanced immunity in fish can be ensured through effective defense mechanism includes lysozyme activity, phagocyte activity, complement activity and bactericidal activity of macrophages (Gantner et al., 2003; Herre et al., 2004; Raa, 2000). The present study, farmers stated that they have got remarkable positive impacts on fish health (reduced disease incidence) due to the use of vitamins in their farms compared to the other immunostimulants. It might because of the essential role of vitamins on fish physiology including growth, resistance to infections, wound healing, response to stressors and possibly lipid metabolism. In addition, Vitamin C is a co-factor in many biological processes including collagen synthesis and cellular functions related to neuromodulation, hormone and immune systems (Mastan, 2015). Ultimately, the use of different types of immunostimulants is becoming an effective means to increase the immune competency and disease resistance in both fish and shellfish cultures. But the lack of knowledge among farmers about the selection and application of these immunostimulatory products has been identified as a remarkable problem in this study.

CONCLUSION

Immunostimulants has been emerging as the most promising prophylactic treatment for the farmed fish. These days, the use of immunostimulants in aquaculture has become an immense potential, but series of focused research is necessary to capitalize on this issue. The present study has been performed only as a baseline field investigation based on the farmers' perception and experiences regarding the use of immunostimulants in Mymensingh district. To get a holistic picture on this aspect in aquaculture, further in-depth and detailed research work should be conducted to find out the efficacy, actual composition and appropriate method of administration for aquaculture immunostimulants in greater Mymensingh as well as in Bangladesh.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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