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Bacterial Disease Management Strategy for Fish Farms of Bangladesh

Bangladesh has emerged as one of the leading nations in aquaculture production during recent years, currently the country stands third in terms of total aquaculture production. Aquaculture (Fig. 1) has evolved as the fastest growing food-producing sector providing employment, income and nutrition to millions of farmers, input providers and consumers in Bangladesh. Mainly exotic fish like *Pangasius hypophthalmus, Oreochromis niloticus* and *Anabas testudineus* and a few indigenous catfish viz., *Clariasbatrachus, Heteropneustes fossilis* and *Ompok pabda* are grown by fish farmers in both monoculture and polyculture practices. These are now considered as successful intensive aquaculture species in the country due to relative easy in culture, high market demand and suitability to local climate conditions. However, intensive aquaculture with these species often causes water pollution problems and diseases. Outbreaks of fish diseases have become one of the major limiting factors in aquaculture production of Bangladesh. Among different types of diseases, bacterial infections are becoming serious problems in aquaculture which are aggravated by poor biosecurity management. Habitual use of drugs, chemicals and antibiotics is leading to another set of problems like bacterial resistance and undesirable residual effects on human health and the environment. The issue of fish health management is, therefore, of considerable importance in order to prevent and manage bacterial diseases in aquafarms. This KGF sponsored project was designed to determine the status of biosecurity in different parts of Bangladesh, isolate and identify fish pathogenic bacteria collected from diseased fish, study efficacies of available prophylactic and therapeutic agents and their practical delivery methods under field conditions, and develop, validate and disseminate a model of fish health management for disease-free sustainable production in intensive aquafarms using cost-effective inputs.

Fig. 1. A typical aquafarm in Bangladesh

Methodology

The study was carried out between December 2017 and November, 2023 in some commercial aquafarms located in Mymensingh, Cumilla and Khulna Divisions of Bangladesh. Data were

collected through questionnaire interviews and participatory rural appraisal tools like focus group discussion (FGD) from 36 selected and 36 other random aquafarms located in three divisions (*i.e.* Mymensingh, Cumilla and Khulna) of Bangladesh.

A set of questions was prepared in a sequential and required logical format to collect data. Boundary fence, foot and tire bath, sanitation and hygiene, pond embankment condition, pond drying, driveways, and shower dress change facilities were observed to assess biosecurity measures.

Diseased fish samples (n= 223) were collected from the affected farms and handled aseptically during disease outbreaks. The samples were preserved following standard methods and brought to the Fish Disease Lab, BAU, Mymensingh for further studies. Isolation, identification and characterization were done to assess the virulence and pathogenicity of bacterial pathogens.

Resistance of the pathogens to antibiotics was studied by the disk diffusion method. Finally, gene sequencing of the bacterial isolates was confirmed by amplifying and sequencing of the 16S ribosomal DNA and species-specific PCR followed by the analysis of nucleotide sequences done in South Korea, which was confirmed by the MEGA X Software.

Fig. 2, 3. Mass culture of bacteria (left) and DNA quantification (right)

Prophylactic trials were carried out with commercial gut, soil and water probiotics. Growth, production and survival of five popular fish species namely, *pangas, shing*, tilapia, *magur* and *koi* in earthen pond culture conditions were recorded. Fifteen ponds, each 0.75 decimal in size, were used for 5 treatments each with three 3 replications. Healthy *pangas, shing, tilapia, magur* and *koi* fingerlings were collected from a hatchery near the BAU campus. The stocking densities were 450/d, 550/d, 650/d, 550/d, and 650/d for *pangas, shing, tilapia, magur* and *koi,* respectively. The fish were fed with a commercial feed supplemented with probiotics. Three commercially available probiotics viz., Zymetin (gut probiotic), pH FIXER (water probiotic) and Super PS (soil probiotic) were used to prepare feed treatments for the experiment: T_1 (soil probiotic), T_2 (gut probiotic), T_3 (combination of soil, gut and water probiotics), T_4 (water probiotic) and T_5 (basal diet only) was the control treatment. After probiotic mixing, feeds were dried overnight inside the room using a fan and were stored in airtight plastic bags in the laboratory at room temperature to use for daily feeding. The fish were fed with the experimental diets three times daily at 0900, 1300 and 1700 hrs, at a rate of 10% initially and then up to 3% of fish body weight. The fish were sampled from each treatment pond fortnightly using a cast net and their body weights measured.

Samples for histology, haematology and bacteriological studies were taken fortnightly. In all the investigations histological changes of gut and liver, haematological, bacteriological and water quality parameters studies under different probiotic supplementations were carried out

by following standard protocols at the Fish Disease Laboratory, BAU, Mymensingh. Training and workshop were arranged for fish farmers of Mymensingh, Cumilla and Khulna regions.

Results and Outputs

No foot bath or tire bath facilities were found in Cumilla and Mymensingh; in Khulna only 4 farms (8.33%) had shower facilities for a complete head-to-toe shower as a part of biosecurity practice. Most of the farmers (80%) measured water quality parameters, had proper feed storage facilities, disinfected fish before stocking and disposed the dead fish regularly. Restrictions against cattle grazing and visitors' movements were strongly practiced (85%) in the Cumilla region, but these restrictions were less in the Mymensingh region (60%). Covering ponds with nets to prevent birds was a common practice (90%) in Cumilla and Mymensingh regions, but not in the Khulna due to larger pond and *gher* sizes. Disinfection of equipment was a common scenario in all divisions. Some catfish (*H. fossilis*) and Thai koi (*A. testudineus*) were found to be diseased. Clinical symptoms like abrasions, deformities, reddish-white lesions and loss of mucus were recorded in *H. fossilis* during the winter months, January-March. Disease symptoms like abrasion, lesion, deep reddish lesion and ulcers were observed throughout the fish body of *A. testudineus* in March in the Cumilla region. Fish organs had more pathogens in private fish farms than those in Government fish farms.

About 100 fish pathogenic isolates were collected from infected fish farms. Biochemical analyses identified 23 pathogenic bacterial strains of *Aeromonas hydrophila, A. veronii, Bacillus cereus, Staphylococcus equorum, Providencia alcalifaciens, Enterococcus odoratus,* and *Acinetobacter rudis*. The most virulent bacteria were screened by the experimental infection of *H. fossilis, M. cavasius, O. pabda, P. hypohphlalmus,* and *A. testudineus*. The bacterial isolates were identified up to the species level using the 16rDNA technique. Antibiogram (Fig. 3) studies using the disk diffusion method revealed ampicillin-resistant strains of A. veronii, B. cereus, and A. rudis isolated from hemorrhagic lesions of *M. cavasius, H. fossilis* and *P. hypophthalmus*. A phylogenetic tree was constructed involving approximately 750 bp sequences of 22 specimens. The genetic distances were calculated using the neighbour joining method in the unit of the number of base substitutions per site. The sequences were into three clusters.

In the prophylactic trials, water quality parameters were favourable. Net weight gain, % of weight gain, specific growth rate, protein efficiency ratio were the higher for the combined probiotics treatment (T_3) followed by the gut (T_2), water (T_4) and soil probiotic (T_1) treatments; the lowest values were obtained for the control (T_5).

The liver of fish in the control ponds (T_5) had pathologies like vacuums, haemorrhage and necrosis and almost normal structures were observed in combined probiotics treated fishes (T_3) followed by the gut (T_2), water (T_4) and soil probiotic (T_1) treated fishes. The T_3 fed fish had increased villi length (VL), enterocyte height (EH) and fold length (FL) followed by T_2, T_4 and T_1.

Hematological parameters *viz.*, WBC and RBC were increased in T_3-fed fish followed by T_2, T_4 and T_1 which indicated a higher immune response compared with the control fishes (T_5). The load of beneficial gut bacteria was the highest for which also indicated the beneficial effects of combined (gut, water and soil) probiotics. Gut probiotic (T_2) and water probiotic (T_4) had also increased gut bacterial loads individually compared with control (T_5).

In the training programs, sessions were arranged related to the importance of biosecurity, probiotics and fish disease prevention in aquaculture. The participating farmers shared their practical problems and experts and guests provided necessary suggestions.

Expected Impact

The project research findings are expected to impact fish health management in aquaculture in that farmers can follow biosecurity measures, use probiotics, take necessary preventive and treatment measures to prevent the outbreaks of bacterial fish diseases. This would result in sustainable and healthy aquaculture production in the country. Moreover, indiscriminate use of chemicals and drugs would be minimized making aquaculture more environment friendly and safer as a major source of protein for the people of Bangladesh.

Recommendations

- Biosecurity issues of aquafarms should be strictly taken care of
- Good Aquaculture Practice (GAP) should be strictly followed
- Indiscriminate use of aqua drugs and chemicals should be halted
- Banned and toxic chemicals which are health hazards should be strictly prohibited
- Waste management should be environmentally friendly
- Application of combined probiotics is recommended for healthy and disease-free fish
- Assistance from Government, NGOs and related stakeholders should be strengthened
- Training should be provided to fish farmers on the latest aquaculture technology

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This Technical Bulletin has been prepared on the basis of technical information available from a completed BKGET-KGF Funded CGP Project, the details of which are given below:

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