Aquaculture is most promising, viable and fast growing enterprise to provide nutritional security and its intensification is required to keep pace with surging need of animal protein which is accompanied with increase in the stress level in the animal as well as the environment. Disease outbreak is considered as most important constraints to its continued expansion. Antibiotics and chemotherapeuants as management therapies result in development of resistant strains, increment in cost inputs and environmental pollution. Consequently, probiotics prophylaxes and therapies are extensively used as environment friendly approach to improve activity of gastrointestinal microbiota, disease resistance, survival, feed utilization growth performance and immune status. Moreover, sound nutrition and adequate feeding demands new species-specific commercial and economically viable diets to support the aquaculture (fish farming) industry. Soybean can act as plant protein resource in feed formulations but is typically low in methionine and its phosphorus is present in bounded form. Duckweed has strong potential to provide highly nutritious, cost effective and environment friendly feedstuff to intensive and extensive culture systems as is rich in essential amino acids including methionine and trace minerals such as phosphorus. Present studies were therefore conducted to study the immunomodulatory and growth promoting effects of gut isolated probiotic Bacillus coagulans in Catla catla, its optimum levels in diets with soybean and duckweed as protein sources. The results of these studies are summarized, experiment wise in this chapter.

Experiment-1

Evaluation of probiotic properties, immunomodulatory effects and optimum inclusion level of probiotic bacterium Bacillus coagulans in formulated feeds for Catla catla.

The isolated probiotic bacterium Bacillus coagulans from the gastrointestinal tract of Catla catla was first assessed for its probiotic properties viz., antagonism towards pathogen and cell surface adhesion. Bacillus coagulans showed antagonistic effect as clear zone of 19±0.9 mm against Aermonas hydrophila and hydrophobicity of this strain of B. coagulans was 30.49±0.84 % in xylene and 22.79±3.96 % in toluene; clearly revealing that this strain can colonize is the gut of Catla catla and has properties of successful probiotics.
In this experiment five dietary treatments (DC, D1, D2, D3 and D4) were performed and fish were fed on approximately isonitrogenous diets (40% protein) for 90 days. In treatment 1 (DC), fish were fed on artificial diet with processed soybean without supplementation of probiotic bacterium *Bacillus coagulans*. In treatment 2 (D1), and treatment 3 (D2) fishes were fed on artificial diet prepared by incorporation of probiotics bacterium *Bacillus coagulans* in proportion of 1000 cells g$^{-1}$ and 2000 cells g$^{-1}$ of feed respectively. In treatment 4 (D3), fishes were fed on artificial diet containing probiotics in proportion of 3000 cells g$^{-1}$ and with proportion of 5000 cells g$^{-1}$ of feed in treatment 5 (D4). Results have revealed that significantly (P < 0.05) high growth performance in terms of live weight gain, growth percent gain in body weight (BW), specific growth rate, gross conversion efficiency and protein efficiency ratio was observed in fishes fed on diet D3 (containing probiotic bacterium *Bacillus coagulans* in proportion of 3000 cells g$^{-1}$ of feed) and also, low feed conversion ratio (FCR), low excretion of metabolites (N-NH$_4$ and o-PO$_4$) and significantly (P < 0.05) high apparent protein digestibility (APD) digestive enzyme activities (protease, amylase and cellulase was found in the treatment D3 when compared with other dietary treatments (DC, D1, D2, D4), indicating better dietary nutrient utilization in the group of fishes fed on diet supplemented with probiotics @ 3000 CFU g$^{-1}$ of feed. With further increase in probiotics supplementation in dietary treatment D4 (probiotics @ 5000 CFU g$^{-1}$) a reduction in growth performance was observed which may be due to low digestibility and production of less enzymes thereby low nutrient utilization. Carcass composition also indicated high accumulation of protein in dietary treatment (D3) indicating better nutrient retention. Postprandial patterns of metabolite excretion revealed that peak values of total ammonia excretion occurred approximately after sixth and twelfth hour after post feeding while o-PO$_4$ production showed an initial high level at 2h post feeding and second peak at 8h post feeding. These results have demonstrated that the inclusion of probiotic bacterium *Bacillus coagulans* in proportion of 3000 CFU g$^{-1}$ of feed is most significant for growth, digestibility and survival of fish, *Catla catla*.

The total erythrocyte and leukocyte count was significantly higher (P<0.05) in D3 (2.4 ±0.08 and 50.5±2.1) than in the control treatment DC, *i.e.* (1.33±.03 and 20.7± 0.82), indicating the better health status of fish because of its role in nonspecific or innate immune and inflammatory responses. The post-challenge data shows increase in leukocyte count irrespective of the *Bacillus coagulans* inclusion and signify a possible increased infection and inflammatory response mediated by leukocyte against bacteria.
Phagocytic ratios, phagocytic indices and NBT assay depicted significantly (P<.05) high values in the fish fed with varying proportion of probiotic *Bacillus coagulans* than control fish during the assay period with maximum values in dietary treatment D3 (containing *Bacillus coagulans* @ 3000 CFU g\(^{-1}\) of diet). Treatment D2 and D3 fed groups showed significantly (P<0.05) higher relative per cent survival, *i.e.*, 86.36 and 90.9 respectively, after challenge trial with *Aeromonas hydrophila* indicating the improved immune response of the fish against the pathogenic bacteria.

**Experiment-2**

**Effect of replacement of processed soybean with duckweed on growth performance, digestibility and nutrient retention in *Catla catla* fingerlings.**

In this experiment, five dietary treatments (TC, T1, T2, T3 and T4) were performed with three replicates of each treatment (Table-3). In treatment 1 (TC), fishes were fed on artificial diet containing processed soybean (*i.e.*, control diet). In treatment 2 (T1), 3 (T2), 4 (T3) and 5 (T4), fishes were fed on duckweed (*Lemna sp.*.) based artificial diets where soybean was replaced by dried duckweed @ 25%, 50%, 75% and 100%. All these diets were isocaloric and isoproteic with approximately 40% proteins.

The results indicated that growth and digestibility parameters such as live weight gain, growth per cent gain in body weight, specific growth rate, gross conversion efficiency and protein efficiency ratio were significantly (P<0.05) higher in treatment T3 however, FCR values were significantly (P<0.0) low in dietary treatment T3 (1.56±.09), containing soybean replaced with duckweed at 75% indicating the better dietary nutrient utilization in this group. Intestinal digestive enzyme activities were significantly (P<0.05) higher in treatment T3 in comparison to control. The Carcass composition also indicated high accumulation of protein in dietary treatment (T3) indicating better nutrient retention. Significantly (P<0.05) low values in total ammonia excretion and reactive phosphate production (mg Kg\(^{-1}\) BW d\(^{-1}\)) were recorded in diet T3 where fishes were fed on duckweed based artificial diet replacing soybean @ 75% as a protein supplement, indicating low levels of pollution in holding water in this treatment. This can be attributed to better dietary utilization of proteins and phosphorus and therefore, low levels of ammonia excretion and orthophosphate production in the holding water. Profit index also revealed significantly (P<0.05) high values in treatment T3 fed group of fishes, which corresponds to low cost of duckweed based diets and higher per cent increases in weight gain. These results suggest that duckweed has potential to replace plant protein soybean upto 75% and can help in reducing the cost of formulated diets for carp fishes.

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Experiment 3

Immunomodulatory and Growth promoting effects of inclusion of probiotic bacterium *Bacillus coagulans* in diet containing duckweed as major protein source for *Catla catla*.

For assaying the effect of incorporation of probiotic bacterium *B. coagulans* in the formulated feeds containing duckweed on growth and associated nutritional physiological changes in *Catla catla*, four dietary treatments (CC, C1, C2 and C3) were performed with three replicates of each treatment and fish were fed on approximately isonitrogenous diets (40% protein) for 90 days. The experimental diets were prepared by replacing 75% of soybean with Duckweed (dried duckweed @ 199.5 g kg\(^{-1}\) of diet) and *Bacillus coagulans* was added at varying proportion. In treatment 1 (CC), fish were fed on artificial diet containing duckweed (199.5 g kg\(^{-1}\) of diet replacing 75% of soybean with Duckweed) without supplementation with probiotic bacteria. In treatment 2 (C1), fishes were fed on artificial diet prepared by incorporation of probiotic bacterium *Bacillus coagulans* in proportion of 1000 cells g\(^{-1}\) of feed. In treatment 3 (C2), fishes were fed on artificial diet containing probiotics in proportion of 3000 cells g\(^{-1}\) of feed and with proportion of 5000 cells g\(^{-1}\) of feed in treatment 4 (C4).

The results revealed that the growth of fish in terms of weight gain, growth/day in percentage body weight, and SGR were significantly (P<0.05) high in treatment C2 where fishes were fed on diet containing probiotics *Bacillus coagulans*, in proportion of 3 ×10\(^5\) cells 100 g\(^{-1}\) (3000 cells g\(^{-1}\)) of feed in comparison to dietary treatments CC, C1, C2 and C4. Also, significantly (P<0.05) high values of digestibility parameters *viz.*, APD, GCE, PER significantly (P<0.05) lower FCR (1.56±0.03) was observed in the dietary treatment C2 while highest was recorded in control group (2.6±0.09) indicating the better utilization of nutrient in this treatment.

Intestinal digestive enzyme activities for protease, amylase and cellulase were significantly (P<0.05) higher in all the dietary treatments in comparison to controls. The values showed an increasing trend from treatment CC to C2 (containing probiotics *B. coagulans* @ 3000 cells g\(^{-1}\)of feed) thereafter, with further increase in the inclusion level of probiotic bacteria (Diet-C3-containing containing probiotics *B. coagulans* in proportion of 5000 cells g\(^{-1}\)of feed) the values decreased indicating that *B. coagulans* @ 3000 cells g\(^{-1}\)of feed is optimum to be incorporated in diets for *Catla catla*.

Fish carcass protein (%), gross energy (kJ g\(^{-1}\)) were found to be significantly (P<0.05) higher in the carcass of fish fed on diet C2 (containing probiotics *Bacillus coagulans* in proportion of 3000 cells g\(^{-1}\) of diet)
Post-prandial excretory patterns of ammonia and reactive orthophosphate in the holding water revealed significantly (P<0.05) low values in total ammonia excretion and reactive phosphate production (mg Kg$^{-1}$ BW d$^{-1}$) in fish fed on diet C2 supplemented with 3×10$^5$ cells 100 g$^{-1}$, i.e., 3000 cells g$^{-1}$ of feed. Irrespective of the protein level and source, N–NH$_4$ excretion showed two peak values, one at 4 h and second at 12 h post-feeding, while o-PO$_4$ production showed an initial high level at 2 h post-feeding and a second peak at 10 h post-feeding.

The increase in erythrocyte and leukocyte count at significant level in C2 (1.94 ±0.04 and 46.8 ± 1.4) than in the control treatment CC (1.37±.06 and 19.5± 0.82 respectively), could be attributed to the fact that probiotic bacterium Bacillus coagulans at its optimum levels enhances the blood parameter values as a result of haemopoetic stimulation.

Phagocytic activity (PA) and phagocytic index (PI) of fish fed on treatment C2 (82.21±1.58 and 3.09±0.06) were significantly higher (P < 0.05) than those fed on the control diet for 90 days. This could be attributed to the fact that different components of bactericidal compounds are released by Bacillus coagulans at its optimum level which activates macrophages of the immune system and that can lead to phagocytosis. There was significant gain in the NBT activity from CC to C2 and thereafter decreased in C3 fed groups revealing that the Bacillus coagulans enhances the immune response at its optimum concentration (3000 cells g$^{-1}$ of diet).

After challenge trial of Catla catla with pathogenic Aeromonas hydrophila, highest relative per cent was recorded in fishes of treatment C2 whereas lowest in fishes of treatment CC (control). This might be due to the enhancement of the non specific immune system of the fish by Bacillus coagulans and mortalities (%) were observed significantly (P<0.05) higher (70±5.7) in control group than in treatment C2 (6.7±3.3).

These results have demonstrated that the inclusion of probiotic bacterium Bacillus coagulans in proportion of 3000 cells g$^{-1}$ of feed is most significant for growth, digestibility and survival of fish Catla catla irrespective of the dietary treatments and duckweed incorporation at 199.5 g Kg$^{-1}$ of feed in treatment T3 appears to be cost effective and promotes growth performance in comparison to complete soybean based diets.

A comparison of data of best diet of experiment 1 (Diet D3) and Experiment 3 (Diet C2) clearly reveal that the parameters on which growth of fish depends, i.e., APD, FCR, Intestinal enzyme activities, phagocytic ratio, phagocytic index, NBT activity were almost same when B. coagulans was incorporated @ 3000 CFU g$^{-1}$ of feed in diets containing soybean @ 266.5 g kg$^{-1}$ or duckweed @ 199.5 g Kg$^{-1}$ of feed.
Salient Findings

1. *Bacillus coagulans* FGB CC1 strain (MTCC no. 9872) isolated from the gut of *Catla catla* can be regarded as autochthonous bacterial strain with probiotic properties as it shows surface adherence and antagonistic activity against pathogenic *Aeromonas hydrophila* and therefore it can be incorporated as additive in diet.

2. It is possible to maintain artificially the gut isolated *B. coagulans* and it can be mass cultured under laboratory conditions.

3. *B. coagulans* when fed with artificial diet can colonize in the gut and built up persistent population in gastrointestinal tract of *C. catla*.

4. The supplementation of *B. coagulans* to 40 % plant protein based formulated diet (Soybean or Duckweed) at 3000 CFU g$^{-1}$ of feed appears to be optimum and could be used to enhance digestibility and health status thereby, improving growth performance of *Catla catla*.

5. Duckweed can be used as soybean replacer in the diet of *C. catla* upto 75% without compromising fish growth and water quality.

6. Incorporation of Duckweed in diets of *Catla catla* can be considered as a cost effective approach reducing the use of comparatively expensive soybean.

7. Better growth performance and nutrient retention requires the reduction of discharge (N-NH$_4$ and o-PO$_4$) in holding water. The use of probiotics bacterium *B. coagulans* and duckweed also alleviates the pollution problems associated with intensive aquaculture system.

8. It was found that specific activity of digestive enzymes can be increased with the incorporation of probiotics at optimum level which may be due to better dietary protein utilization by colonization of probiotic bacteria and its exogenous enzyme production.

9. *Bacillus coagulans* has immunomodulatory properties as it enhance non specific immune response, i.e., phagocytic and activity and NBT assay in dietary treatments of Experiment 1 and 3 in comparison to their control groups.

10. After 10 days of challenge trial with *Aeromonas hydrophila* highest mortality was recorded in control diets w.r.t. *Bacillus coagulans* supplemented diets in dietary treatments of Experiment 1 and 3.

11. Results of present studies would form the basis for further research which is required to elucidate the usefulness for commercial application in fish production.
Therefore, such diets when commercialized may reduce the disease risk, enhance the profit index, improve the immune response of the fish, alleviate the pollution problem of holding water and will be environmental and user friendly. However, the experiments need to be conducted at field to check the expected outcomes before commercialization.