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Role of Probiotics in the Feeds for Long-Whiskered Catfish, *Sperata aor* (Hamilton, 1822)

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Abstract

The present study was undertaken to assess the role of artificial diet containing probiotic on growth performance and survival rate of aquaculturally important candidate species, *Sperata aor* (Hamilton, 1822) under laboratory conditions. Three dietary treatments, T1 (live feed *Eisenia foetida*), T2 (40% plant protein) and T3 (probiotic *Bacillus* sp.) were maintained in triplicate with 15 fingerlings each for the duration of 90 days. Studies revealed that significantly ($p<0.05$) higher growth performance was observed in fish fed on diet T3 with probiotic as compared to the others. No significant differences were observed in protein efficiency ratio (PER) and gross conversion efficiency (GCE) while feed conversion ratio (FCR) was low for T3 indicating efficient utilization of the feed. The results were further confirmed by analyzing the water samples for total ammonia and reactive phosphate which revealed significantly ($p<0.05$) low values in fish fed on diet T3 incorporated with *Bacillus*. Rhythmic pattern of excretion with peak values of total ammonia were found after 6 h of feeding while peak values of total reactive phosphate found after 4 h post feeding while decreased gradually being low for T3 fed group. These results concluded that low values of ammonia and reactive orthophosphate help us to manage water pollution in holding water. Incorporation of probiotics such as *Bacillus* in the diets of *S. aor* has additional advantages which can enhance growth, survivability and improves water quality.

Keywords: *Sperata aor*, *Bacillus*, Fish feed, Growth performance, Probiotics

1. Introduction

Fishery development is gaining attention of fishery scientists as intensive fish culture system is being developed in the country to meet the massive demand by optimal utilization of potential resources. Intensive culture of carnivorous fishes on live food entails high production costs (Nandeesha et al., 1990). Formulated diets can minimize the cost involved and allow the production of advanced juvenile carnivorous fishes (Bromley, 1980) by weaning from live food to moist and dry diets. Weaning is mainly affected by diet palatability, feeding strategy, initial fish size and cannibalism. For success in fish culture, in addition to quality feed and good environment we must ensure disease free fingerlings. Antibiotics have sometimes been used to reduce disease; however, indiscriminate use has in some cases led to increased antibiotic resistance and problem of tissues residues and trade issues. Thus, to solve this and improve effective farm management use of 'probiotic' is one such boon to aquaculture (Bandyopadhyay, 2004; Richard et al., 2007; Bhatnagar and Saluja, 2019; Bhatnagar and Dhillon, 2019; Bhatnagar and Rathi, 2020). Probiotics are cultured products or live microbial feed supplement which beneficially affects the host by improving its intestinal balance and health of host. *Bacillus* sp. are most commonly used probiotic bacterium in aquaculture (Bandyopadhyay, 2004; Sumathi *et al*., 2014).

The long-whiskered catfish, *Sperata aor* (Hamilton, 1822) is the largest species of the family Bagridae and can attain a length of six feet or more. Although, it has great medicinal value, less bone content and high delicious flavors of fish flesh, yet farmers take least interest in culture of this candidate species, because of its feeding and eating habits, and slow growing nature. Studies have revealed that the natural food of this species can successfully be replaced by the use of soybean with 40% protein content (Bhatnagar et al., 2018). However, development of diets which can further reduce the excretion level may help in achieving better growth and nutrient retention. Raparia (2014) reported significantly high growth performance in *Catla catla* fed with diet containing isolated bacteria *B. coagulans*. Bhatnagar et al. (2012) evaluated gut isolated probiotic bacteria as biotechnological tool in enhancing the growth performance and digestibility in fish. Numerous studies have shown that the incorporation of probiotics can enhance the growth efficiency, weight gain and efficient conversion of feed into flesh in fish (Daniels et al., 2010; Bhatnagar and Raparia, 2014; Bhatnagar and Lamba, 2017), however, role of probiotic in the feeds of *S. aor* has not been studied so far. Therefore, the present study was conducted to study the usefulness of incorporation of probiotic bacterium *Bacillus* in the plant protein-based diet of *S. aor*.

2. Materials and Methods

The present study was conducted at Aquaculture Research Unit, Department of Zoology, Kurukshetra University, Kurukshetra, Haryana (India). Three dietary treatments, T1 (live feed *Eisenia foetida*), T2 (40% plant protein) and T3 containing probiotic (*Bacillus* sp.) were maintained for evaluating their effect on the growth of the fingerlings. *E. foetida* were cultured in the vermiculture unit of Department of Zoology, Kurukshetra University, Kurukshetra. *Bacillus* sp. was procured from Macro-Pro

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Hyderabad and the feed containing 40% protein was formulated, prepared (Bhatnagar et al., 2018) and probiotic was incorporated by spraying its suspension. All the ingredients were thoroughly mixed for making dough using distilled water and passed through a mechanical pelletizer to obtain pellets (0.5 mm thick) which were oven-dried after air drying. The probiotic diet was prepared by spreading the feed in the sterile tray and the incorporation was achieved by spraying the probiotic bacteria *Bacillus* sp. (1000 CFU g^{-1}). The air-dried feed was stored in vacuumed plastic containers at 4ºC in refrigerator. Ingredient and proximate composition have been shown in Table 1.

Sperata aor (Family: Bagridae) fingerlings (fish weight ranging from 0.71 to 0.77 g) were procured from a local fish farm and acclimated under laboratory conditions after disinfecting with potassium permanganate solution for two weeks prior the feeding trial in plastic tub containing (50 L capacity) 30 L of chlorine free tap water where temperature at 25±1.0°C was maintained. Fingerlings were initially fed with basal diet to allow acclimation to the system prior to the initiation of experiment. To maintain hygienic condition and prevent water deterioration caused by remaining feed and feces, the plastic tubs were cleaned daily prior feeding time in morning by siphoning out the excreta and 80% of the water was exchanged to prevent sudden increase in water temperature because the experiment was conducted in summer months.

All the experimental groups with 15 fingerlings each in triplicate were fed on respective feed for 60 days at 3% body weight in two installments at 08:00 and 15:30 h. Ration was adjusted by weighing the fingerlings fortnightly after initially weighing at the beginning of the trial. The uneaten feed and fecal matter were siphoned out, stored for subsequent analysis. Length of fish was measured using a simple centimeter scale. Various growth parameters were calculated for evaluating dietary performances and nutritional indices (Garg et al., 2002) (where, W1= Initial Weight (g), W2 = Final Weight, t = Days of experiment)

- Weight gain (g) = W_2-W_1
- Per cent Growth rate:

$$\text{Per cent Growth rate} = \frac{W_2 - W_1}{W_1} \times 100$$

- Specific Growth Rate (SGR):

$$\text{SGR} = \frac{\ln W_2 - \ln W_1}{t} \times 100$$

- Feed Conversion Ratio (FCR):

$$\text{FCR} = \frac{\text{Feed offered (Dry weight) (g)}}{\text{Body weight gain (Wet weight) (g)}}$$

- Gross Conversion Efficiency (GCE):

$$\text{GCE} = \frac{\text{Body weight gain (Wet weight) (g)}}{\text{Feed offered (Dry weight) (g)}}$$

- Protein Efficiency Ratio (PER):

$$\text{PER} = \frac{\text{Wet weight gain (g)}}{\text{Crude protein fed (\%)}}$$

The proximate/biochemical analysis of fish carcass was carried out at the start and termination of the feeding trial following AOAC (1995).

For maintaining the proper water quality, the water samples from all the experimental tubs were collected during experimentation and analyzed for dissolved oxygen (DO), pH, total ammonia (NH_4-N) and orthophosphate following APHA (1998). At end of the feeding trial, fingerlings were offered same diet in sufficient quantity. After 2h, excess feed was removed and fixed levels of water were maintained for experiments. Water samples from each tub were collected at 2h intervals to estimate the excretory levels of total ammonia (N-NH_4) and reactive orthophosphate (o-PO_4) following APHA (1998), and were calculated following Sumagaysay-Chavoso (2003). The quantity of nitrogen and phosphate excreted by fish in holding water was calculated as follows:

$$\text{Total N} - NH_4/o - PO_4 \text{ excretion (mg kg}^{-1}\text{BW day}^{-1}) = \frac{[(N - NH_4/o - PO_4)_{120} - (N - NH_4/o - PO_4)_0]}{\text{Fish biomass kg}^{-1}} \times a$$

$(N\text{-}NH_4/o\text{-}PO_4)_0$ and $(N\text{-}NH_4/o\text{-}PO_4)_{120}$ = Concentration at times 0 and 120 min

a = amount of holding water (L)

Significant differences among the dietary treatment groups were tested by Analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan, 1955) for experiments. Statistical significance was settled at a probability value of $p<0.05$. All statistics were performed using SPSS Version 18.0.

3. Results and Discussion

The growth responses of the fingerlings fed on the different experimental diets are shown in Table 2. The growth of fish in terms of weight gain (g), % growth rate and SGR were significantly ($p<0.05$) high in the T1 and T3 fed group. Digestibility parameters GCE and PER were not much different but FCR was found to be low in T3 fed group incorporated with probiotic *Bacillus* clearly indicating the efficient utilization of the feed. No significant differences were observed in survival rate between both the treatments. Although similar growth was observed in T1 (live feed) and T3 (probiotic) but low FCR makes the incorporation of probiotics efficient for *S. aor* fingerlings. The higher growth performance may be due to direct growth promoting effect of probiotics by direct involvement in nutrient uptake. Bandyopadhyay (2004) have demonstrated that probiotics act as agents which serve to modify or manipulate the microbial communities in water such that they reduce or eliminate selected pathogenic microbes and improve the growth of target species. The results further depicted low FCR and high PER values in the diets with probiotic. This may be due to enhanced digestion through the supply of essential enzymes. Bandyopadhyay (2004) have shown various ways through which probiotic agents could act in

Table 1. Ingredient and proximate composition (% dry weight basis) of the different diets

Dietary Treatments	T1 (*Eisenia foetida*)	T2 (Soybean)	T3 (*Bacillus*)
Ingredient composition			
Ground nut oil cake	-	650	650
Rice bran	-	37	37
Wheat flour	-	37	37
Soybean*	-	266	266
Bacillus (CFU g^{-1})	-	0	1000
Mineral mixture**	-	10	10
Proximate composition			
Moisture (%)	-	7.18 ± 0.03	7.59± 0.22
Crude Protein (%)	-	40.06 ± 0.27	40.12± 0.23
Crude Fat (%)	-	9.01 ± 0.10	8.80±0.20
Crude Fiber (%)	-	6.60 ± 0.06	6.50± 0.06
Total Ash (%)	-	5.75 ± 0.03	5.57±0.10
Nitrogen free extract (%)	-	30.62 ± 0.23	30.54±0.18
Gross energy (kJ g^{-1})	-	18.31 ± 0.03	18.43±0.95

*Soybean was hydrothermically processed in an autoclave at 121°C (15 lbs for 15 minutes) to eliminate anti-nutritional factors (Garg *et al*. 2002).
**Each kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114g, iron 979 mg, zinc 2 mg, iodine 15 mg, DL-methionine 1.920 g, L-lysine monohydrochloride 4.4 g, calcium 30%, phosphorous 8.25%.

Table 2. Growth performance of the fingerlings fed on the experimental diets

Growth parameters	T1 (*Eisenia foetida*)	T2 (Soybean)	T3 (*Bacillus*)
Initial weight (g)	0.72 ± 0.01^{A}	0.71 ± 0.02^{A}	0.77 ± 0.01^{A}
Final weight (g)	2.08 ± 0.01^{A}	1.89 ± 0.06^{B}	2.12 ± 0.03^{A}
Weight gain (g)	1.36 ± 0.02^{A}	1.18 ± 0.03^{B}	1.35± 0.02^{A}
Survival rate (%)	96.2±0 ± 3.80^{A}	94.24 ± 2.98^{B}	96.20 ± 3.80^{A}
% growth rate	188.80 ± 2.78^{A}	166.19± 2.34^{B}	175.32 ± 1.80^{A}
Specific growth rate (SGR)	2.27 ± 0.02^{A}	1.95 ±0.07^{B}	2.24 ± 0.02^{A}
Feed conversion ratio (FCR)	2.01± 0.07^{A}	2.02 ± 0.01^{A}	1.94± 0.02^{B}
Gross conversion efficiency (GCE)	0.20 ± 0.15^{A}	0.22 ± 0.00^{A}	0.20 ± 0.15^{A}
Protein efficiency ratio (PER)	-	0.03 ± 0.00^{A}	0.04 ± 0.00^{A}

All values are Mean ± S. E. of mean.
Means with different letters in the same row are significantly ($p<0.05$) different (Duncan's Multiple Range test).

Table 3. Proximate carcass composition of the fingerlings fed on experimental diets

Proximate composition	Initial value	T1 (*Eisenia foetida*)	T2 (Soybean)	T3 (*Bacillus*)
Moisture (%)	82.5 ± 0.26^{A}	77.57 ± 0.30^{B}	77.24 ± 0.22^{B}	78.70 ± 0.25^{B}
Crude protein (%)	12.23 ± 035^{B}	16.33 ± 0.15^{A}	13.67 ± 0.07^{B}	15.16 ± 0.07^{A}
Crude fat (%)	1.89 ± 0.65^{B}	2.65 ± 0.27^{B}	4.17 ± 0.09^{A}	2.14 ± 0.08^{B}
Total ash (%)	2.03 ± 0.14^{B}	2.48 ± 0.18^{B}	3.14 ± 0.12^{A}	2.28 ± 0.18^{B}
Nitrogen free extract (%)	1.23± 0.11^{A}	1.70 ± 0.04^{A}	1.48 ± 0.14^{A}	1.56 ± 0.15^{A}
Gross energy (kJ g^{-1})	3.65 ± 0.32^{C}	4.74 ± 0.13^{B}	5.06 ± 0.65^{A}	4.86 ± 0.03^{B}

aquaculture systems. These include competitive exclusion of pathogens, enhancing digestion through the supply of essential enzymes, moderating and promoting the direct uptake of dissolved organic material and active promotion of pathogen inhabiting substances.

Crude protein (%), crude fat (%), total ash (%) and gross energy (kJ g^{-1}) were found significantly ($p<0.05$) different in the carcass of fish fed on diets (Table 3). Crude protein (%) value of probiotic diet (T3) fed group was similar to live feed T1 while significantly ($p<0.05$) higher as compared to those of soybean fed group T2 with significantly ($p<0.05$) high crude fat (%). There were no significant ($p<0.05$) differences among moisture, and NFE while gross energy was significantly ($p<0.05$) high in *Bacillus* incorporated diet. Similar results supporting the fact of improving growth and development of fish with the inclusion of probiotics have been demonstrated by various studies and role of probiotics in health management of fish revealed good responses (Gopalakannan et al., 2001; Bairagi et al., 2004; Nayak and Mukherjee, 2011; Bhatnagar et al., 2012; Bhatnagar and Raparia, 2014; Dhillon, 2018).

The dissolved oxygen (DO) fluctuated between 4.5±0.02 to 4.6±0.01 mg L^{-1} and pH remained alkaline (7.3±0.03 to 7.4±0.02) (Fig. 1). Ammonia and phosphate are the two excretory products that draw in the consideration as they frame the significant source of water pollution caused by feed and fish. The protein present in the dietary feedstuff

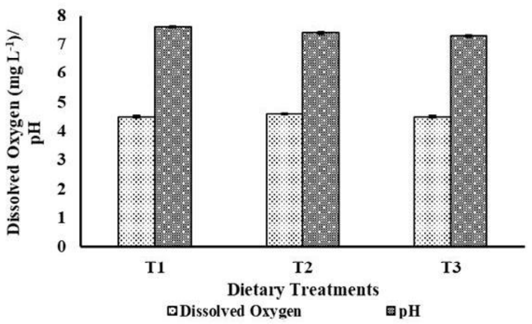


Fig. 1. Effect of different dietary treatments on dissolved oxygen and pH

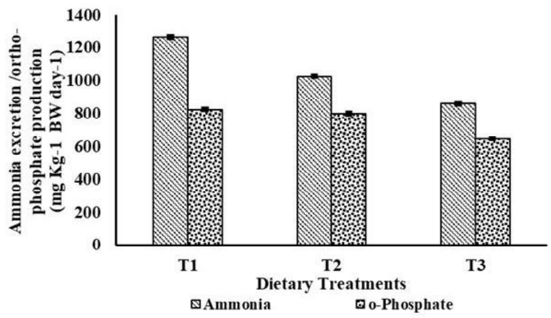


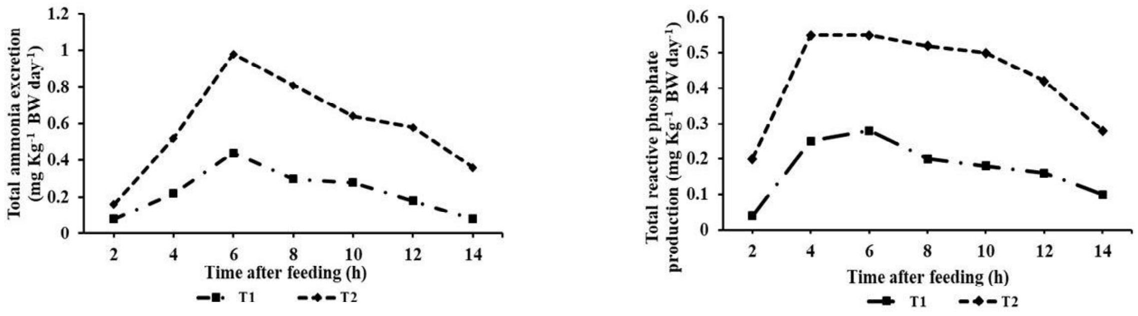
Fig. 2. Total ammonia excretion and o-phosphate production (mg Kg-1 Body weight per day) for *Sperata aor* fingerlings in different dietary treatments

of fish feed that cannot be digested and assimilated by the fish body form the part of the metabolic waste to be excreted out in the surrounding water. In general, significantly ($p<0.05$) high values in total ammonia and reactive phosphate excretion (mg Kg^{-1} BW d^{-1}) were recorded in T3 where fingerlings were fed on diet without *Bacillus* (Fig. 2). Rhythmic patterns of ammonia excretion and reactive phosphate production were observed with peak values in N-NH_4(total ammonia) excretion occurring approximately after 6 h of feeding and peak values in o-PO_4 (reactive phosphate) production was observed after 4 h of feeding for T1 and T2 but after 6 h for T3, which gradually declined thereafter and remained low for T3 fed group (Fig. 3).

It is evident from the result of this experiment that incorporation of probiotic *Bacillus* in the diet of *S. aor* not only enhanced growth performance but also improved the water quality. Many investigations have been carried out by scientists who revealed that the composition of the intestinal flora of the larvae from first feeding onwards play an important part in the defense against colonization and growth opportunistic pathogens. A decrease in the total ammonia excretion and phosphate production was also low in the treatment when fish were fed on a diet containing probiotic bacterium. The reason might be optimization of nitrification rates to keep low ammonia concentration, optimization of denitrification rates to eliminate excess nitrogen, and maximizing carbon mineralization to carbon dioxide to minimize sludge accumulation. Dong et al. (2000) has also reported similar bioremediation effects of probiotics in the diets and demonstrated that there are various ways through which probiotics act as bioremediation agents in the aquaculture systems.

Boyd and Gross (1998) found that bacterial (*Bacillus* sp.) additions in the pond did not improve water quality as expected. They, however, observed higher survival of fish in ponds treated frequently with live *Bacillus.* Result of the present studies also depicted high survival rate in the fishes fed on diets with probiotics. However, improvement in the water quality characteristics i.e. low ammonia and phosphate (mg kg^{-1} body weight) and higher DO concentration also coincided with high growth and survival in the present studies. Suhendra et al. (1997) concluded that probiotics can act as bioremediation agents in aqua/shrimp culture ponds, but their efficacy depends on understanding the nature of competition between species or shares of bacteria. Bidhan and Bandyopadhyay (2002) reported similar results while using selected strains of *Bacillus* sp. in shrimp and carp culture ponds, respectively.

It can be concluded that these probiotics can act as bioremediation agent but not much is known about the condition under which they may be effective, their application, rates and methods. However, the products are safe to humans and environment and their use poses no hazard (Boyd and Gross, 1998) therefore, further studies should be conducted with their incorporation especially in the culture system where slow growing carnivorous fishes are cultured and they will prove an important break through to improve the environment sustainability of aquaculture.



A B

Fig. 3. Post prandial excretory patterns of A. ammonia and B. o-phosphate (mg Kg-1 Body weight per day) in holding water for *Sperata aor* fingerlings in different dietary treatments

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4. References

AOAC 1995. Official methods of analysis. Association of Official Analytical Chemists Incorporation. Arlington, USA, 684 pp.

APHA 1998. Standard methods for the examination of water and waste water. 20th ed. American Public Health Association, New York.

Bairagi, A., Sarkar Ghosh, K., Sen, S.K. and Ray, A.K. 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquac. Res., 35 (5): 436-446.

Bandyopadhyay, P. 2004. Improvement of growth, nutritional quality and immunity of Indian major Carps through probiotic supplement, Ph.D. thesis, Vidyasagar University, Midnapore- 721102, West Bengal, India, 292 pp.

Bhatnagar, A. and Lamba, R. 2017. Molecular Characterization and Dosage Application of Autochthonous Potential Probiotic Bacteria in *Cirrhinus mrigala*. *J. Fisheries Sciences,* 11 (2): 046-056.

Bhatnagar, A., Dhillon, O. 2019. Characterization, screening and application of bacteria with probiotic adequacy isolated from the gut of *Labeo calbasu* (Hamilton, 1822). *Fisheries and Aquat. Life*, 27 (4): 178-189.

Bhatnagar, A. and Raparia, S. 2014. Optimum dietary inclusion level of *Bacillus coagulans* for growth and digestibility improvement for *Catla catla* (Hamilton). *Int. J. Curr. Res. Rev*., 6 (7): 1-10.

Bhatnagar, A., Raparia, S. and Kumari, S. 2012. Isolation and Influence of *Bacillus coagulans* CC1 on growth performance and digestive enzyme activities of *Catla catla* (Hamilton). *J. Nat. Sci. Sustainable Tech.*, 6 (3): 237-253.

Bhatnagar, A. and Saluja, S. (2019) Synergistic effects of autochthonous probiotic bacterium and Mentha piperita diets in *Catla catla* (Hamilton, 1822) for enhanced growth and immune response. *Journal of Fisheries and Aquatic Sciences*, 22(1):1-16.

Bhatnagar. A., Sushma, and Dhillon, O. 2018. Evaluation of dietary protein levels for the growing *Aorichthys aor* (Hamilton 1822). *Int. J. Zool. Stud*., 3 (2): 188-193.

Bhatnagar, A. and Rathi, P. 2020. Dosage Determination of Autochthonous Probiotic Bacterium Aneurinibacillus aneurinilyticus for the Optimum Growth and Immunostimulation of *Labeo calbasu* (Hamilton, 1822). Annal. of Biol., 36 (1): 81-87.

Bidhan, C.P. and Bandyopadhyay, P. 2002. Probiotics can assure nutritional security in aquaculture. An overview. In: Kumar, A. (eds.), Ecology and Ethology of Aquatic Biota. 2nd ed. Daya Publishing House, New Delhi, India, pp. 220-225.

Boyd, C.E. and Gross, A. 1998. Use of probiotic for improving soil and water quality in aquaculture pond. Abstracts of the 5th Asian Fisheries Forum. 11-14 November, 1998, Chiang Mai, Thailand.

Bromley, P.J. 1980. The effect of dietary water content and feeding rate on the growth and food conversion efficiency of Turbot *(Scophthalamus maximus*). *Aquaculture,* 20: 90-99.

Daniels, C.L., Merrifield, D.L., Boothroyd, D.P., Davies, S.J., Factor, J.R. and Arnold, K.E. 2010. Effect of dietary *Bacillus* sp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. *Aquaculture,* 304: 49-57.

Dhillon, O. 2018. Evaluation of dietary protein requirements and role of intestinal probiotic bacteria for growth promotion in *Labeo calbasu* (Hamilton 1822). Ph.D. Thesis, Kurukshetra University, Kurukshetra, Haryana, India.

Dong, H., Fredrickson, J.K., Kennedy, D.W., Zachara, J.M., Kukkadapu, R.K. and Onstott, T.C. 2000. Mineral transformation associated with the microbial reduction of magnetite. *Chem. Geol.,* 169: 299-318.

Duncan, D.B. 1955. Multiple range and Multiple F Tests. Biometrics, 11 (1): 1-42.

Garg, S.K., Kalla, A. and Bhatnagar, A. 2002. Evaluation of raw and hydrothermically processed leguminous seeds as supplementary feed for the growth of two Indian major carp species. *Aquac. Res*., 33: 151-163.

Gopalakannan, A., Nowsheen, J., Ramya, S. and Arul, V. 2001. Biocontrol of *Aeromonas Hydrophilia* using lactic acid bacteria. National Work Aquaculture. Medicine, School of Environment. Studies Cochin University of Science and Technology, Cochin, Kerala, 68-69.

Nandeesha, M.C., Srikanth, G.K., Keshavanth, P., Varghese, T.J. and Shetty, H.P.C. 1990. Growth performance of *Cyprinus carpio communis* fed on diets containing different levels of deoiled silkworm pupae. In: Hirano, R. and Hanyu, I. (eds.), The Second Asian Fisheries Forum, Asian Fisheries Society, Manila.

Nayak, S. and Mukherjee, S. 2011.Screening of gastrointestinal bacteria of Indian major carps for a candidate probiotic species for aquaculture practices. *Aquac. Res.,* 42: 1034-1041.

Raparia, S. 2014. Use of Potential Probiotic Bacteria *Bacillus coagulans* and Duckweed in feeds for growth promotion and Immunomodulatory Effects in *Catla catla*. Ph.D. Thesis, Kurukshetra University, Kurukshetra, Haryana, India.

Richard, A.S., Chhorn, L., Mediha, Y.A. and Philips, K.H. 2007. Effects of probiotic bacteria as dietary supplements on growth and disease resistance in young channel catfish, *Ictalurus punctatus* (Rafinesque). J. Appl. Aquaculture, 19 (1): 81-91.

Suhendra, T., Handokoa, J., Octaviano, D., Porubcan, R.S. and Douillet, P.A. 1997. Management with bacterial probiotics for *Vibrio* and virus control in an Indonesia prawn. In: Alsto, D.W., Green, B.W. and Clifford, H.C. (eds.), Proceedings IV. Central Amercian Aquaculture Symposium: Sustainable Culture of Shrimp and tilapia. 201-202.

Sumathi, C., Dillibabu, V., Madhuri, Dash-Koney, MohanaPriya, D., Nagalakshmi, C. and Sekaran, G. 2014. Dietary inclusion of protease producing novel *Pontibacter spp.* and *Bacillus megaterium* as a Probiotic enhances immune responses in *Labeo rohita*. Pak. *Journal of Biological Sciences,* 17: 451-461.

Sumagaysay-Chavoso, N.S. 2003. Nitrogen and phosphorus digestibility and excretion of different-sized groups of milkfish (*Chanos chanos* Forsskal) fed formulated and natural food-based diets. *Aquac. Res.,* 34 (5): 407-418.