ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effects of probiotics on the survival and growth of Catla catla, Labeo rohita, Cirrhinus mrigala and Pangasius hypophthalmus under polyculture system

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Manuscript Info

Manuscript History:

Received: 18 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

Polyculture systems, Probiotics, Survival and Production.

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Abstract

Aquaculture is one of the fastest growing food sector of the world and accounts for almost 50% of the world fish production. Sustainable production is affected chiefly by factors such as poor water quality management,nutritionaly imbalanced supplementary feed and the disease incidence. To control of these diseases, indiscriminate use of antimicrobial drugs in fish farming lead to the emergence of antibiotic resistant bacteria. Probiotics play an important role in growth increment and stress mitigation in fish. In the present investigations two types of commercial probiotics (PVS Labs, Andhra Predesh) were used i.e Amruth (fish) as feed probiotic-treatment-1 and Procon- PS (fish) as water and soil probiotics micro feed as feed probiotic on treatment-2 and control pond without probiotics were selected ponds at Kaikaluru of Krishna district, Andhra Pradesh, India. The ponds are stocked with catla (*Catla catla*), rohu (*Labeo rohita*), mrigala (*Cirrhinus mrigala*) and Pangas (*Pangasius hypophthalmus*) with the stocking ratio of 3: 9: 3: 6. And the culture period goes to one year i.e. August, 2013- July 2014. The results clearly showed that the concentrations of ammonia, nitrite and orthophosphates are low in the experimental ponds compare with control pond. Further the investigations reveal that growth and survival are obtained higher in probiotic treated ponds than that of control pond.

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INTRODUCTION

Fish culture has become an important economic activity in many states of India. In large scale production facilities, where culture organisms are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and results in serious economic losses. Prevention and control of diseases have led during recent decades to a substantial increase in the use of antimicrobial drugs. However, the utility of antimicrobial drugs as preventive measure has been questioned, given extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria (Schwarz *et al.,* 2001; Akinrbowale *et al.,* 2006). Globally, several tones of antibiotics have been distributed in the biosphere during an antibiotic era of only about 60 years duration (Scan, 2003). These amounts of antibiotics have exerted a very strong selection of pressure towards resistance among bacteria, which have adapted to this situation mainly by a horizontal and promiscuous flow of resistance genes (Scan, 2003). Probiotics are beneficial bacteria capable of repressing the growth of pathogenic organisms either through the production of inhibitory substances or by competition (Moriarty, 1998). Regular application of probiotics is done to maintain the desired population of good bacteria and improve water quality. According to Fuller (1987) probiotics as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient

environment". Gram *et al*., (2001) stated that the use of commercial probiotics in fish is relatively ineffective as most commercial preparations are based on strains isolated from non-fish sources that are unable to survive or remain viable at high cell density in the intestinal environment of fish during the active growth phase of the fish. Hence, there is elegant logic in isolating putative probiotics from the host in which the probiotics intended for use. The probiotic application came first as it is environmentally safe and cost effective (Moriarty, 1997). This benefit of probiotics will be long lasting and the application of probiotics will be long lasting, and the application of probiotics will becoming a major field in the development of aquaculture (Ravi *et al.*, 1998). The use probiotics in human and animal nutrition is well documented (Fuller, 1992, Mulder *et al.*, 1997, Rinkinen *et al.*, 2003, Krishna *et al.*, 2009) and they began to be applied in an aquaculture (Gatesoupe, 1999, Gomez-Gil *et al.*, 2000, Verschuere *et al.*, 2000, Irianto and Austin, 2002 a, 2002b, Bachiere, 2003, Krishna *et al*., 2009). Probiotic in fish culture have been shown to have several modes of action; competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of nutrition of host species through the production of supplementary digestive enzymes (Verchuere *et al.*, 2000).

Materials and Methods:

For the experiment was conducted in a private fish farm located in Kaikaluru, Krishna district, Andhra Pradesh, India. Total nine ponds triplicate tanks are taken as control pond and experimental ponds in triplicate. The experiments were conducted for a period of one year from August, 2013- July 2014. The size of the each was one hectare and it is rectangular in shape. Control ponds are denoted as 'C' and experimental ponds denoted as Treatment pond-1 (T1) and Treatment pond-2 (T2). In the experiments, two types of commercial probiotics (PVS laboratories, Andhra Pradesh) were used i.e. Amruth (fish) as feed probiotic in **Treatment-1**, Procon-PS (fish) as water and soil probiotic + micro feed (fish) as feed probiotics in **Treatment-2** and control pond is without probiotics. Water exchange was done 5-10% in every 10 days up to the harvest of the ponds.

Pond preparation:

All the experimental ponds were dewatered and dried for fifteen days before stocking. Purpose of sundried is to disinfect the pond and also the stabilization of pH, liming with CaO was applied at the rate of 125kg/ha with dusting method (Wahab *et al.*, 2002). Essential precautionary measures were taken to screen the water inlets to avoid the entry of exotic fishes and other unimportant material in to the fish ponds. After two weeks of pre stocking management methods, each pond was watered up to 1.5 to 2.0 m and this water level was maintained throughout the experimental period. All the ponds were fertilized with organic manure (cow dung, 2500kg/ha) as started dose to stimulate the productivity of the ponds. Then the ponds were applied with inorganic fertilizers, urea and triple super phosphate at the rate of 12.5 and 6.25Kg/ha respectively.

Stocking of fish species in experimental ponds:

Two weeks after manuring, each pond was stocked with catla (*Catla catla*), rohu (*Labeo rohita*), mrigala (*Cirrhinus mrigala*) and Pangas (*Pangasius hypophthalmus*) with the stocking ratio of 3: 9: 3: 6. The average body weight was recorded at the time of stocking. At the time of stocking, fingerling size (length of the fish) was taken by centimeter scale and weight of the fish was measured by electronic balance.

Water quality parameters:

Water quality parameters such as temperature, transparency, dissolved oxygen, pH was measured daily and total alkalinity, hardness and ammonia were measured fortnightly.

Fertilization and supplementary feed:

After stocking of fish species, daily the amount of organic manure, inorganic fertilizer and supplementary feed was calculated on N-equivalence of 0.2 g N/100 g body weight of fish. In this experiment, all the experimental ponds received the same quantity of Nitrogen. The supplementary feed was supplied twice in a day at morning 8.00 am and evening 6.00 pm. The feeds were calculated at the rate of 5-7% of the body weight.

Fish harvesting:

At the end of the experiments all the fishes were harvested by netting and drained the pond. In each pond fishes were counted species wide. The final length (cm) and weight (g) gained by each species was recorded. The survival rate of each species from control tank and experimental tanks were calculated from the number fishes of species were harvested during harvesting time. Following growth parameters are studied during experiment

1. **Specific growth rate:**

$$SGR = \frac{\text{In (Final wet body weight)} - \text{In (Initial wet body weight)}}{\text{Time duration (days)}} \times 100$$

2. **Feed conversion ratio (FCR)**

$$FCR = \frac{\text{Total feed fed (kg)}}{\text{Total wet weight gain (g)}}$$

3. **Survival rate:**

At the final harvesting the survival rate of four species was calculated by the following Formula

$$\text{Survival rate} = \frac{\text{Number fishes recovered}}{\text{Number of species stocked}} \times 100$$

4. **Total fish production under different treatment:**

At the end of the experiment, the total harvested fishes of four species were weighed to calculate the total fish production under probiotic and control ponds.

Results:

The results of water quality parameters in different parameters in control and experimental ponds such as temperature, transparency, dissolved oxygen, pH, total alkalinity, total hardness and ammonia were monitored during the study period were shown in Table 1 and production survival details are given in Table 2. The survival, growth and production details of catla, rohu, mrigala and pangas in the control and experimental ponds were given in Table 2. The better performance in experimental ponds was quite evident. The application of probiotics proved its worthiness through ways of better pond environment. In the experimental ponds treatment-2 (T2) goes to better results compare with treatment-1 (T1). The yellow and green colonies, which are noticed in the experimental pond initially, were eliminated after use of probiotics while such colonies increased in the control ponds. Further ammonia level was below the detectable level in the experimental ponds.

Discussion:

In culture systems, chemotherapeutic agents such as commercial antibiotics (chemicals) are commonly employed for disease management, although this is not advisable due to high cost, environmental hazards, and the antibiotic resistance developed by many pathogens (Kruse and Soram, 1994). Diseases that may results from pathogenic bacteria are prevented or reduced by the use of probiotic, which make the fish more resistant (Olsson *et al.,* 1992; Tannok, 1998). Probiotics are also some times expected to have a direct growth promoting effect on fish either by a direct involvement in nutrients uptake or by providing nutrients or vitamins (Ringo and Gatissoupe, 1998). It has also been demonstrated experimentally that probiotics indeed my enhance growth of fish (Noh *et al.,* 1994; Bogut *et al.,* 1998; Krishna *et al*., 2009). In the present experiments probiotics enhance the production of experimental ponds compare with control ponds. Application of probiotics was found to improve the water quality parameters also the condition in the pond bottom there by enabling the successful culture and harvest. Use of probiotics in the supplementary diets of fish culture during grow out phase is useful in polyculture system. They can be introduced into the culture environment to control and compete with pathogenic bacteria and to promote the growth of culture organisms. Further, probiotics are non-pathogenic and non-toxic organisms without undesirable side effects when administered to aquatic organisms (Farzanfar, 2006).

In order to overcome fish diseases, scientists have selected certain beneficial microbes, to be used as feed additives. Probiotic bacteria can enhance the host enzyme secretion which increases the digestive efficacy of the complex protein and lipids included in the diet thus, increasing food digestion and absorption by the host (Tover *et al.,* 2002; Ghosh *et al.,* 2007, 2008). Probiotic bacteria also affect the production of the vitamins particularly B group vitamins (Goldin and Gorbach, 1992; Ghosh *et al.,* 2007). Hence the higher survival rate could be linked to the intestine probiotic bacteria which produce vitamin B-complex. Thus revealed that probiotic incorporated diets helped to increased survival and growth performance of the experimental fish. Probiotics can also be considered as microbes to improve the nutritive value of an animal feed (Ireanto and Austin, 2002a). Until recently, one of the most frequent procedures used to avoid the establishment of undesirable bacteria in a target organism was the administration of antibiotics in the water. Probiotics are presently used in fish culture to modify and manipulate microbial population in the pond and environment and to reduce or eliminate selected pathogen species of micro-

organisms leading to better growth and survival of the culture species (Irianto and Austin, 2002 a, b). The present study results indicate improved survival and growth of fishes were observed in the experimental ponds with supplementary feed and by using probiotics. Successful probiotic bacteria are able to colonize in intestine, at least temporarily by adhering to the intestinal mucosa. The adhesive probiotic bacteria could prevent the attachment of pathogens, such as *coliform* bacteria and *clostridia* and stimulate their removal from the infected intestinal tracts (Lee *et al.,* 2000;). Probiotics are bio-friendly agents, they can be introduced into the culture environments to control and compete with pathogenic bacteria as well as promote the growth of culture organisms. Several studies have attributed probiotic effect to competition for energy sources (Furazanfar, 2006; Subhash *et al.,* 2007; Krishna *et al.,* 2009; Mohideen *et al.,* 2010; Cristia *et al*., 2014; Divya *et al*., 2014; Janardhan reddy *et al*., 2015).

In the present experiments with probiotics ponds gave better production and water parameters such as pH also is maintained at optimal range. Thus probiotic was found to use full in maintaining the pond water pH a desired level (Sambasivam *et al.,* 2003). In culture practice, water quality deteriorates mainly due to accumulation of metabolic wastes. This could be due to the degradation of organic matter facilitates nutrients recycling and competes with other pathogenic bacteria (Sanders *et al*., 2003). Feed probiotics and water probiotics also improved the health of fishes to some extent but water quality parameters were much improved in T1 and T2 than control. Among the treatments T2 goes to better survival and high yield. Similar results observed by Abasali and Mohmad (2011).

Table 1: Water quality parameters in culture tanks treated with probiotics

Parameters	Control pond	Treatments	
	C	T1	T2
Temperature(^{0}c)	28.5 (28.0-31.0)	29.0 (28.0-31.0)	29.0 (28.0-31.0)
Transparency (cm)	50 35-55)	40.5 (30-45)	30.2 (25-35)
Dissolved oxygen (ppm)	4.5 (3.0-5.5)	5.2 (4.5-5.8)	5.8 (5.0-6.0)
pH (ppm)	7.8 (7.6-8.0)	7.9 (7.6-8.0)	7.9 (7.6-8.0)
Total Alkalinity (ppm)	120 (100-140)	100 (80-110)	110 (80-120)
Total Hardness (ppm)	145 (110-160)	140 (110-150)	135 (100-140))
Ammonia (ppm)	1.25 (0.75-1.50)	0.69 (0.5-1.0)	0.35 (0.25-0.50)

Table 2: Growth, Survival and Production of fishes in different probiotic treatments

Parameters	Species	Control pond	Treatment pond	
			T-1	T-2
Initial Weight (g)	Catla	15.6	16.2	16.3
	Rohu	16.2	15.9	16.4
	Mrigala	25.4	22.9	25.1
	Pangas	15.6	15.8	15.1
Final Weight (g)	Catla	955.6	1055.6	1250.6
	Rohu	985.4	1150.4	1355.9
	Mrigala	915.6	1065.2	1123.5
	Pangas	996.7	1255.8	1564.9

Specific growth rate (%)	**Catla**	2.480	2.499	2.550
	Rohu	2.461	2.542	2.573
	Mrigala	2.116	2.219	2.172
	Pangas	2.495	2.580	2.705
Survival rate (%)	**Catla**	50.5	70.6	76.8
	Rohu	58.2	76.8	82.6
	Mrigala	54.4	70.8	86.8
	Pangas	51.6	72.9	84.6
Total Production (Kg/Ha/Year)	**Catla**	1206.4	1863.1	2401.2
	Rohu	4301.3	6626.3	8399.8
	Mrigala	1245.2	1885.4	2438.0
	Pangas	2571.5	4577.4	6619.5

In present study the application of only water and soil probiotics are directly applied the tank culture that reducing the organic matter loads. *Bacillus* sp of bacteria are reported to more efficiently improve water quality. *Bacillus* also reduced the quantity of ammonia, nitrite in the water Skjermo and Vadstein, (1999). In the present study, application of water probiotic significantly reduced the levels of ammonia in experimental ponds than control ponds. Among all the treatments T2 goes to better results i.e. in fish weight, survival and yield also. The application for probiotics in fish culture ponds appears bright. There is an over increasing demand for fish production and a similar increase in the search for alternative to antibiotics. The application of probiotics intended for culture system now attracting considerable attention and number of commercial products is available, particularly directed at the culture organisms. Probiotics strains already adapted through natural process to the dynamics of an aquaculture. Production system will probably lesson farm management environmental manipulation practices are required to active the desired antibiotic effect in final product (Kesarcodi-Watson *et al.*, 2008). Bidhan *et al*.,(2014) concluded that the probiotic status and mode of action in the intensive aquaculture. Mohapatra *et al* .,(2015) reported that the pond trails are necessary to implement multispecies probiotic enhanced and nutritionally balanced diets in commercial tropical fish farms. Introducing such specially intended probiotics is bound to favor an increase in the application of probiotics particularly areas of fish culture in view of global market ability. Krishna *et al.*, (2009) explained that the probiotic application in biological systems in details and further they explain sustainable aquaculture management.

It is essential to understand mechanisms of action in order to define selection of criteria for potential probiotics. More information on the host/microbe interaction are still needed for better understanding of the composition and functions of the indigenous micro biota, as well as microbial culture of probiotics. The decision of using probiotics in fish culture has been in large part of a result of historical and empirical use and not based on scientific criteria. The use of probiotics is an important management tool, but its efficiency depends in understanding the nature of competition between species and strains. In the present study the experimental ponds were better survival and significant growth was observed for fish fed with supplementary feed with probiotics. General selection criteria are mainly determined by bio-safety consideration methods of production and processing, methods of administration and of probiotics and location of the body where micro-organisms are expected to be active and not harmful to the host. Further investigation on these lines would throw more light in to the actual mechanism of probiotic system in fishery section.

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