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Effect of Probionts with different feed additives on growth in Pearl spot, *Etroplus suratensis*

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Abstract

World aquaculture is the fastest growing food-producing sector in the world. Globally, aquaculture is expanding into new directions, identifying and diversifying. With increasing demand for environment friendly aquaculture, the use of alternatives of additives and probiotic growth promoters in fish nutrition is new widely accepted. Science-based knowledge on probiotics has increased in recent years. As natural additives such as banana peel and potato peel have much potential to increase the efficiency and sustainability of aquaculture production. This review summarize the evaluates current knowledge of the potential application and challenges of probiotics in aquaculture practice.

Keywords: Probiotics, potato peel, banana peel, aquaculture.

Introduction

In recent years, there is a great interest in the use of probiotic bacteria in aquaculture to improve disease resistance, water quality and growth of farmed fish (Verschuere *et al*., 2000). Application of probiotics is an important tool in disease control and growth promoters in fishes. *Bacillus* has been studied as an organism that could enhance the role of gut microflora and as a barrier against pathogens (Sogaard and Jessen, 1990).

In the natural environment agricultural and aquaculture waste materials are available in large quantities. These materials are not wastes but waste resource, because they contain nutrients at varying level. A variety of substances, including many waste materials from the agriculture, food and rendering industries are recycled into feed for food-producing animals (Hao *et al*., 2006; Krishna, 1999). The agricultural wastes such as banana peel, potato peel and sugarcane bagasse are grouped under carbohydrates and cellulose rich materials.

In the present study, an attempt has been made to investigate the probiotic effects of extracellular enzyme producing microflora *Bacillus* isolated from fish gut of *E.suratensis* and feed additives, on the growth of *E.suratensis*, was measured after rearing period.

Materials and Methods

A healthy *E.suratensis* weighing 15.51±0.31g were collected from Rajakkamangalam estuary, Kanyakumari, TamilNadu. The fish were acclimatized to the ambient laboratory conditions of temperature (28.0±1.0^{o}C) and pH (7.5) for about 2days. They were fed ad libitum on freshly prepared pelleted diets. Fifty percent water exchange was carried out daily. Three

different types of experimental diets supplemented with probionts *viz*, *1*% *Bacillus* +2% Banana peel powder (DietA), 1% *Bacillus* +2% Potato peel powder (DietB) and control diet (DietC) with 2% CMC without probionts were formulated and prepared by using different ingredients. Three sets of experiment were conducted in 50L capacity tanks containing 30L water, each with 6 fishes for a period of 91 days in triplicate. During the experimental period, the prepared diets were fed to the experimental animals two times a day. The water quality parameters were also maintained at the optimum level during the study period.

(1). Growth response

During the experimental period, 30 to 40% water exchange was made daily. Before water exchange, the fecal matter egested was carefully collected daily and were oven dried, weighed and used for further analysis. Considering the amount of uneaten feeds with that of the total feed provided, the amount of feed consumed by individual fish was calculated. At the end of the experiment, the fishes were weighed separately in all the treatments. Performance parameters such as weight gain, specific growth rate and feed conversion ratio of *E.suratensis* at the end of the experimental period were calculated.

(2). Biochemical composition

Then they were sacrificed and the muscle, gill and gut samples were removed under low temperature of 10-12^{o}C at aseptic condition and used for protein, carbohydrate and lipid analysis. The biochemical constituents of experimental fish samples were analyzed using the procedure of Lowry *et al*., 1951; Roe *et al*., 1955; Folch *et al*., 1957 respectively.

(3). Gut microbial analysis

Microbial diversity was carried out in experimental fishes during 0, 10, 30, 60 and 91days of culture period. For screening the bacteria from the digestive tract, fishes was stunned to death and washed several times with sterile sea water to prevent contamination. The gut was removed by aseptic dissection. The sections were homogenized separately in a mortar and pestle by using sterile sea water individually and 10% v/v homogenates prepared. Serial dilutions were made using 9ml sterile

sea water and plated in triplicate into MRS agar. The plates were incubated at room temperature for 24hrs. The bacterial isolates were identified by using the scheme of Bergey's Manual of Determinative Bacteriology.

Results

Growth Response

*E.suraten*sis fed with 40% experimental DietA and DietB had higher growth compared to those fed with control DietC. In fish fed with DietB higher growth (16.46±0.76) was recorded when compared with those fed with DietA and DietC. The food conversion efficiency (FCE) was high (56.91±0.60%) in DietB and DietA than DietC fed fishes. The specific growth rate (SGR) was also higher in DietB fed fishes (0.80±0.62%). The food conversion ratio (FCR) exhibited a low (better) value of 1.70±0.14% in DietB fed *E.suratensis*, when compared to the high FCR value in DietA and Diet C fed fishes (Table 1).

Table 1. Overall growth responses of *E. suratensis* fed on experimental diets (Diet A and B) and control diet (Diet C) during 91 days of feeding experiment.

Parameters	Growth responses		
	Diet A	Diet B	Diet C
Initial wt(g)	15.00±0.20	15.00±0.60	15.00±0.20
Final wt(g)	26.7±0.18	29.2±0.45	25.8±0.75
Production(g)	11.60±0.48	14.20±0.17	10.80±0.26
Food consumed(g)	28.84±0.56	29.70±0.83	38.62±0.86
FCE (%)	42.40±0.23	40.96±0.76	27.96±0.65
SGR (%)	0.59±0.52	0.73±0.27	0.59±0.32
FCR	2.37±0.25^{a}	2.09±0.45^{ab}	3.53±0.23^{a}

Biochemical composition

The overall results on variation in biochemical composition inferred that, it was much influence by both variation in *bacillus* supplementation and also additives. The protein, carbohydrate and lipid content of fish fed on DietB were higher than DietA and DietC. Among the biochemical constituents analyzed in the muscle, gill and gut samples were not differed much between fish fed with DietB and DietA (Table 2). Whereas, this value was not varied much in DietA fed fishes and less in DietC fed fishes.

Table 2. Biochemical composition of muscle, gill and gut samples of *E.suratensis* fed on DietA, DietB and DietC

Bacterial diversity of gut microflora

Biochemical composition	Fish samples								
	Muscle			Gill			Gut		
	DietA	DietB	DietC	DietA	DietB	DietC	DietA	DietB	DietC
Protein	37.65± 0.12	40.96± 0.14	33.46± 0.20	31.17± 0.22	32.45± 0.14	27.15± 0.12	25.23± 0.21	27.36± 0.17	23.36± 0.14
Carbohydrate	3.98± 0.02	4.73± 0.01	3.06± 0.01	3.09± 0.01	3.42± 0.01	2.00± 0.01	2.82± 0.01	3.12± 0.02	2.25± 0.01
Lipid	3.10± 0.01	3.53± 0.02	2.86± 0.02	3.00± 0.01	3.12± 0.02	2.08± 0.12	2.18± 0.01	2.34± 0.01	1.82± 0.01

The experimental fish fed with *Bacillus* with potato peel powder (Diet B) recorded the maximum bacterial population during 91 days of experimental period. Whereas, this value was not varied much in DietA fed fishes and less in control diet (Diet C) fed fishes (Fig 1). The bacterial population further increased on the 91 day in the gut of probiotic supplemented diet fed fishes than control diet fed fishes.

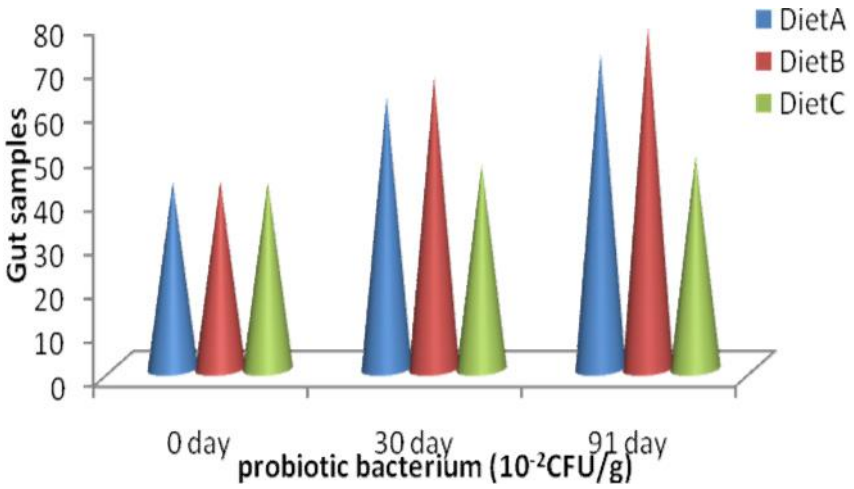

Figure 1. Probiotic bacterial population in gut samples

The generic composition in the selected gut regions of experimental fish varied much with respect to the variation of dietary source as well as experimental duration (Table 3). The bacterial population further increased on the 91st day in the gut of probiotic supplemented diet fed fishes than control diet fed fishes. For instance, in the gut region of *E.suratensis* fed on DietA and DietB on 91 day of experiment, the genera like *Bacillus* spp. was higher than other genera presented were *Klebsiella* sp., *Pseudomonas* sp., *Micrococcus* sp., and *Enterobacter* sp (Fig 2).

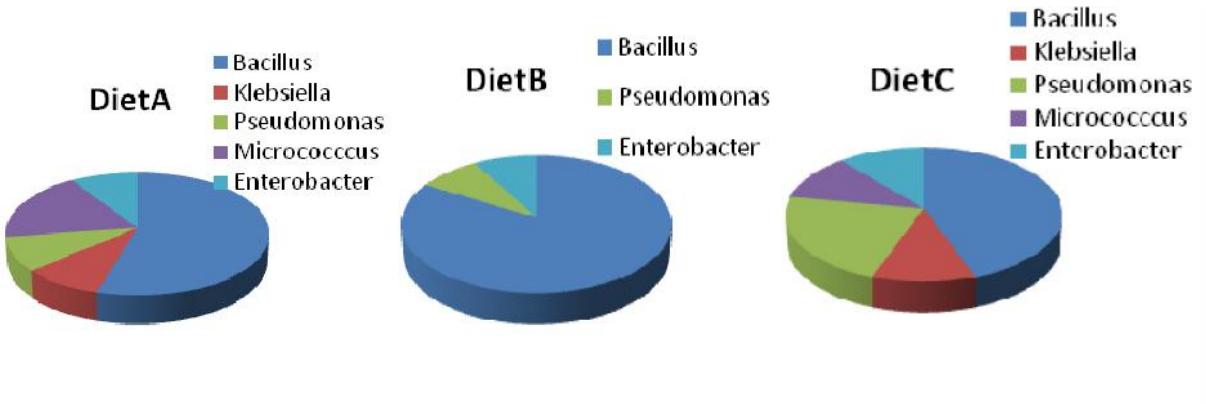

Figure 2. The generic composition in gut regions of experimental fish

Discussion

Probiotics should be normal inhabitants of the host and able to survive and grow at the site of application while exerting their beneficial effect. Increasing with the demand for eco-friendly aquaculture, use of probiotics or prebiotics as immuno stimulants have received heightened attention. Probiotic usage can improve the immunity of cultured animals to pathogenic organisms and induce the growth performance. And also a variety of substances, including many waste materials from the agriculture, food and rendering industries are 'recycled' into feed for food producing animals. This work explored the possibility of using cellulose rich vegetable waste food staffs banana peel, potato peel and sugarcane bagasse in fish feed and also their efficiency in added diets.

Francis *et al* (2001) inferred that adding potato peel powder can still support the good results in the final fish weight. They further indicated that the nutritional quality of potato peel meal as determine by growth indices was adequate and in terms of survival. In consistence with this, in the present work the highest percentage weight gain was recorded in fish fed with diets containing potato peel meal. These results are in agreement with those of Oyrin Olukunle (2006) was also reported that sweet potato meal was the best growth feed additives for fish production. The overall results indicated the fact that banana peel incorporated feed exhibited the second best performance. Aarumugam *et al*., (2013) reported that the performance of vegetable wastes incorporated feed, particularly banana peel on survival, growth, nutrition and general health of fish was appreciable.

Incorporating supplements such as probiotics has been practiced with the aim of enhancing weight gain within a short period of time. Gomez *et al*., (2008) reported that the presence of *bacillus* sp. as a supplement in shrimp feed significantly improved the weight gain during 45 days of experimental duration. Phianphak *et al*., (1999) reported that a mixture of *lactobacillus* sp. isolated from chicken gastrointestinal tracts had improved the growth and survival rates of fish, when these strains were fed for duration of 100 days. Quiet interestingly; in the present work also addition of additives and probiotics along with the fish diets enhanced the weight gain in fish *E.suratensis* when fed for duration of 91 days. Moreover, Rosovitz *et al*. (1998) concluded that administration of *bacillus* bacteria to trout resulted on enhanced digestion of food and improved growth, including a better food conversion ratio (FCR), high specific growth rate (SGR) of experimental fishes. Similarly, the present work also documented a better FCR, SGR in fish fed on DietB when compared to the control DietC fed fishes.

Sogaard and Jessen (1990) reported that introduction of probionts particularly *Bacillus* results in variation in the gut microbial composition. These changes in relation to the flora's capacity to prevent infections must be regarded as a favourable increase of the *lactobacillus* concentration, reduction of *E.coli* and as increase the levels of organic acids. In the present work also, the same phenomena was observed in *bacillus* strains added experimental diets fed fishes after the feeding duration of 91 days. Furthermore, the experiments showed that *bacillus* species possess a board spectrum of enzymatic activities, which may contribute to the digestibility of a number of feed ingredients such as protein, carbohydrate and lipid.

The bacterial density increased in gut regions of fishes fed with experimental diets after 91 days. This increase in percentage of the microbeas may be attributed to the dietary supplemented probiotic strains. Most probiotics are normal intestinal bacteria,

when administered in effective doses, establish and colonise in the digestive tract and increase the natural flora of the digestive tract. This prevents colonization of pathogenic organisms and helps the optimum utilisation of feed (Fuller, 1997). The present study revealed that *E.suratensis* fed with DietA and DietB showed maximum percentage of *bacillus* groups than DietC after 91days feeding experiment. In the DietC fed fishes, it is evident that the occurrences of *Bacillus* strains were in decreasing order.

The overall results inferred that, the agricultural wastes such as potato peels and banana peels could be efficiently used as an alternate nutrient source in the fish feed and which were supported the colonization of probiotic bacteria in the gastrointestinal tract of *E.suratensis* which in turn enhanced the overall growth responses and biochemical synthesis of the candidate fish species. It would therefore be more economical to utilize the renewable cellulose rich materials of plant origin in fish feed added with appropriate probiotic bacteria, so as to prepare cost effective and eco friendly diet.

Conclusion

The present work proved the effect of various bacterial probionts and vegetable waste on increased growth of *E.suratensis*. The results will be further used in aquaculture industry for large scale production of *E.suratensis* under controlled environmental conditions. Furthermore this work can be extended in aspect of application in various other fishes also using different sources of food waste.

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