Effect of a Probiotic and Herbal Additives on Growth, Survival and Disease Resistance of Striped Murrel

M.A. Haniffa, B. Kavitha Bharathi, I. Viji Margaret and Bilal Ahmad Paray

Among air-breathing freshwater food fishes, snakeheads (commonly called murrels in India) constitute the most common and dominant group. They fetch very high prices because the flesh quality is high in terms of taste and texture, with a low fat content, fewer intramuscular bones, and perceived medicinal value (Haniffa *et al*. 2004). The striped murrel *Channa striata* (Fig. 1) is a promising species for aquaculture in Asian countries but is constrained by the availability of seed and feed. Murrels are carnivorous, piscivorous and cannibalistic in nature.

Murrels are affected by Epizootic Ulcerative Syndrome (EUS), primarily caused by the fungus *Aphanomyces invadans* and secondarily by the bacterium *Aeromonas hydrophila* (Karunasagar *et al*. 1995). Infection by *A. hydrophila* is generally associated with poor environmental conditions (Grabowshi *et al*. 2004). Invasion by the pathogen results in skin lesions that lead to high mortality. Common control measures of the diseases rely on antibiotics, chemotherapeutants and vaccines. However, using antibiotics for treatment is problematic, relating to the acquisition of genes that result in antibiotic-resistant strains of bacteria and residues of antibiotics that ultimately diffuse into the aquatic environment (Ellis 1991, Smith *et al*. 1994). Application of modern tools is necessary to increase disease resistance and promote murrel aquaculture (Ghosh *et al*. 2002).

In the last few years probiotics and herbs have become an integral part of culture prevention for promoting growth and disease resistance. This strategy offers many advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production through enhanced growth and disease preventio. In aquaculture, the range of probiotics evaluated for use is considerably wider than in terrestrial agriculture.

An alternative and safer way to tackle *Aeromonas* infection is an eco-friendly development of resistance by the target species through application of probiotics (Ringo and Birkbeck 1999, Irianto and Austin 2002) and herbs (Guojun *et al*. 2008). These immunostimulants enhance the humoral and cellular immune responses in specific and non-specific ways, thereby reducing the risk of diseases.

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FIGURE 1. C. striatus *affected by the disease Epizootic Ulcerative Syndrome.*

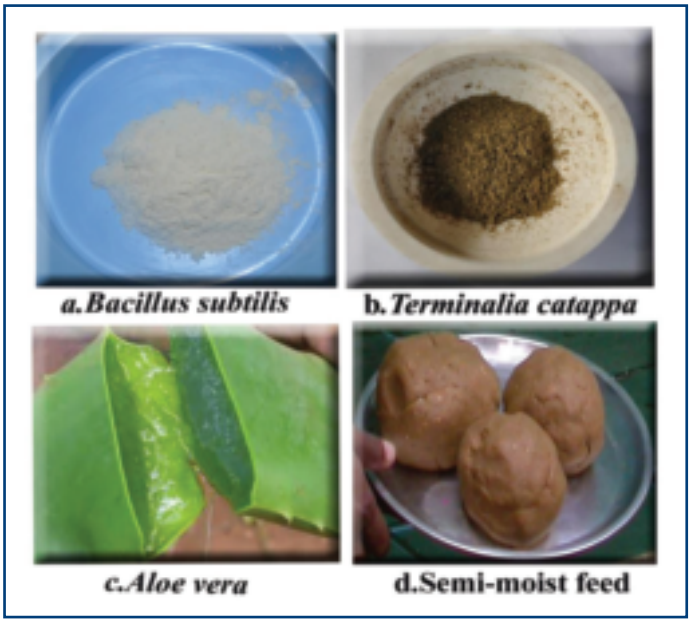
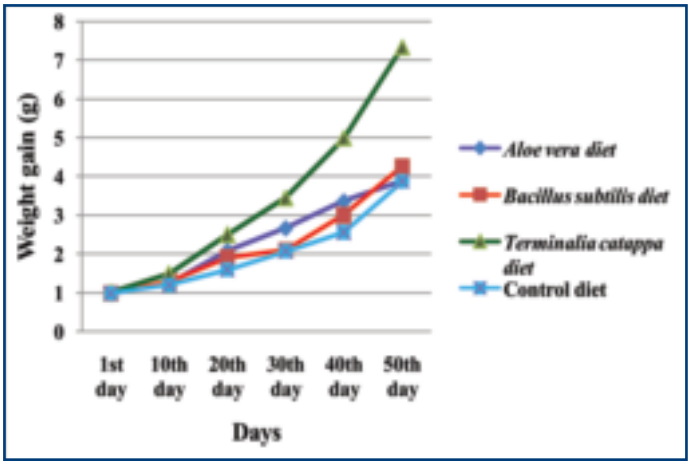
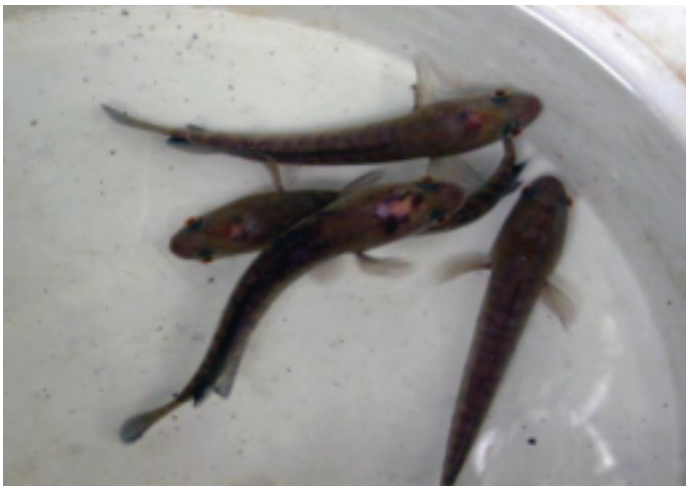


FIGURE 2. *Feed additives and semi-moist feed.*





TOP LEFT, FIGURE 3. *Injection of* A. hydrophila *to* C. striatus *fingerlings.*
TOP RIGHT, FIGURE 4. *Effect of feed additives on weight gain in* C. striatus *fingerlines fedd different feeds.*
LEFT, FIGURE 5. *Fingerlings of C. striatus showing skin lesions challenged by* A. hydrophila.

TABLE 1. SEMI-MOIST FEED FOR *CHANNA STRIATA.*

Ingredient	*%*
Fishmeal, anchovy	26.9
Soybean flour	25
Jawala, Acetes sp.	20
Tapioca meal	10.9
Wheat flour	10
Sunflower oil	5.8
Monosodium phosphate	0.3
Mineral premix	0.5
Aqua Saver	0.3
Vitamin and Mineral premix	0.1
Feed additive	0.2

a Vitamin mixture providing the following concentration per kilogram diet; vitamin A 5000 IU; vit D 400 IU; vit E 20 mg; thiamin mononitrate (B1) 4 mg; riboflavin (B2) 6 mg; nicotinamide 50 mg; pyridoxine hydrochloride 3 mg; calcium pentohenate 10 mg; cyanocobalamine (B12) 2mg; ascorbic acid (vit C) 100 mg; biotin 0.1 mg. b Trace mineral mix use providing the following concentration (ppm) copper 10; iron 100; manganese 50; zinc 50; cobalt 0.05; and iodine 0.1 (Dhanaraj and Haniffa 2011). Feed additives- B. subtilis, T. catappa, A. vera.

The goal of the investigation described here was to assess the effects of a common probiotic bacterium, *Bacillus subtilis*, and herbal additives Indian almond *Terminalia catappa* and aloe *Aloe vera* on growth performance, survival and disease resistance of striped murrel fingerlings challenged by *A. hydrophila*.

Effects on Growth Rate

Four experimental diets were prepared (Table 1). Each of three diets contained one of the following feed additives (Fig. 2): *Bacillus subtilis,* Indian almond *Terminalia catappa* and aloe *Aloe vera*. A fourth (control) diet was prepared without feed additives. Ingredients were mixed with feed additives and autoclaved, semi-moist feeds were prepared and stored at -20 C.

Striped murrel fingerlings (1.0±0.2 g/fish) were obtained from the Centre for Aquaculture Research and Extension (CARE) Aquafarm, St. Xavier's College, Palayamkottai, Tamil Nadu, India and stocked into cement tanks (300 L) filled with groundwater at 10 fingerlings per tank in triplicate for each treatment. Fingerlings were fed 5 percent of body weight daily, divided into three feedings, for 50 days and the feeding rate was adjusted every 10 days. Growth parameters were calculated using standard methods (Adewolu 2008).

Growth of striped murrel fed diets with a probiotic bacterium and aloe were not different from striped murrel fed the control diet. However, growth of striped murrel fed the diet with Indian almond was substantially greater than that of fish fed the control diet (Table 2). Some herbs promote cellular lipid and fatty acid utilization and protein accumulation resulting in good growth performance (Ji *et al*. 2007). The growth increase in rohu *Labeo rohita* fed with herbal supplemented diet was a result of improved food utilization and high protein synthesis (Johnson and Banerji 2007).

Effects of Disease Challenge

A. hydrophila was isolated from diseased striped murrel

(CONTINUED ON PAGE 66)

TABLE 2. GROWTH PERFORMANCE OF *C. STRIATUS* FINGERLINGS FED ON DIFFERENT DIETS.

Parameters	Aloe vera	Bacillus subtilis	Terminalia catappa	*Control*
Initial length (cm)	4.6±0.4	4.7±0.3	4.9±0.4	4.9±0.4
Final length (cm)	7.9±0.5	8.2±0.8	9.7±0.4	7.9±0.5
Initial weight (g)	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2
Final weight (g)	3.9±0.7	3.9±0.7	7.3±0.6	3.9±0.5
Weight gain (g)	2.9	2.9	6.3	2.9
SGR (%/d)	2.7	2.9	4.0	2.6
Condition Factor	0.79	0.77	0.80	0.79
Survival (%)	100	90	90	60

TABLE 3. MORTALITY OF *C. STRIATA* FINGERLINGS CHALLENGED BY *A. HYDROPHILA*.

Diet	*Hours post-challenge*							
	24	*48*	*72*	*96*	*120*	*144*	*168*	*Total*
Control	*	*	*	1/10	*	1/9	2/8	4/10
Terminalia catappa	*	*	*	*	1/10	*	*	1/10
Aloe vera	*	*	*	*	*	*	*	0/10
Bacillus subtilis	*	*	*	*	*	1/10	*	1/10

collected from the wild. A subculture was maintained on tryptone soy agar slopes (Himedia) at 4 C and was routinely tested for pathogenicity. A stock culture in tryptone soy broth was stored at -20 C to provide stable inoculum throughout the study (Dhanaraj and Haniffa 2011). After the 50-d feeding trial, experimental fish were injected intraperitoneally (Fig. 3) with one-day-old culture of *A. hydrophila* (100 µL/fish) at 106 cfu/mL. All fish were observed daily for one week to record clinical signs and mortality.

Survival of striped murrel fed diets supplemented with aloe was greatest (Table 3). Survival of striped murrel fed diets supplemented with *Bacillus* and Indian almond was greater than that of fish fed the control diet. The improved survival suggests that production of metabolites by probiotics suppressed the pathogenicity of *A. hydrophila* (Fig. 5) and ultimately resulted in better survival and growth of the host. Aloe was effective as a disease suppressant and antibacterial agent in striped murrel. Substances in aloe are known to have anti-bacterial and anti-fungal effects (Klein and Penneys 1988, Kim *et al*. 1999, Sunitha 2012). Aloe is recommended as a feed supplement for treatment of EUS.

Notes

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Acknowledgments

Authors acknowledge the financial assistance received from ICAR-NAIP Sub Project entitled, "A Value Chain on Murrel Production in Tamilnadu and Orissa" (F.No.1(5)/2007-NAIP dt. 22 Aug. 2008). We are grateful to Rev. Dr. Joseph S.J., Principal and Consortium Leader, St. Xavier's College (Autonomous), Palayamkottai, for providing the necessary facilities.

References

Adewolu, M.A. 2008. Potentials of sweet potato (*Ipomoea batatas*) leaf meal as dietary ingredient for *Tilapia zillii* fingerlings. Pakistan Journal of Nutrition 7:444-449.

Das, S., L.R. Ward and C.Burke. 2008. Prospects of using marine Actinobacteria as probiotics in aquaculture. Applied Microbiology and Biotechnolog, 81:419-29.

Dhanaraj, M and M. A. Haniffa. 2011. Effect of probiotics on growth and microbiological changes in snakehead *Channa striatus* challenged by *Aeromonas hydrophila*. African Journal of Microbiology Research 5:4601-4606.

Ellis, A.E. 1991. Tissue residues of chemotherpeutants in fish. Bulletin of the European Association of Fish Pathologists 11:22-25.

Ghosh, S., A. Sinha and C. Sahu. 2007. Isolation of putative probionts from the intestines of Indian major carps. Journal of Aquaculture 59(3):127-32.

Grabowshi, L.D., S.E. LaPatra and K.D. Cain. 2004. Systemic and mucosal antibody response in tilapia, *Oreochromis niloticus* (L.), following immunization with *Flavobacterium columnare*. Journal of Fish Diseases, 27:573-581.

Guojun, Yin, Ardo Laszlo, Jeney Zsigmond, Xu Pao and Jeney Galina. 2008. Chinese herbs (*Lonicera japonica* and *Ganoderma incidum*) enhance non-specific immune response of tilapia, *Oreochromis niloticus* and protection against *Aeromonas hydrophila*. Deseases in Asian Aquaculture VI: 269-282.

Haniffa, M.A., K Marimuthu., M. Nagarajan., A.J. Arokiaraj and Kumar, D. 2004. Breeding behaviour and parental care of the induced bred spotted murrel *Channa punctatus* under captivity. Current Science 86:1375-1376.

Haniffa, M.A and Abdul Kader Mydeen. 2011. Hematological changes in *Channa striatus* Experimentally infected by *Aeromonas hydrophila*. Bioresearch Bulletin 4:246-253.

Irianto, A and Austin B. 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 25:333-342.

Ji, S.C., O. Takaoka, G.S. Jeong, S.W. Lee, K. Ishimaru, M. Seoka and K. Takii. 2007. Dietary medicinal herbs improve growth and some nonspecific immunity of red seabream *Pagrus major*. Fisheries Science 73:63-69.

Johnson, C. and A. Banerji. 2007. Influence of extract isolated from the plant *Sesuvium portulacastrum* on growth and metabolism in freshwater teleost, *Labeo rohita* (Rohu). Fishery Technology 44(2):229-234.

Karunasagar, I., G. Sugumar and I. Karunasagar. 1995. Virulence characteristics of *Aeromonas* sp. isolated from EUS-affected fish. Pages 307S–314 *In*: M. Shariff, J. R. Arthur and R. P. Subasinghe, editors. Diseases in Asian Aquaculture Vol. II. Manila, Philippines: Fish Health Section, Asian Fisheries Society,.

Kim, H.S., S. Kacew and B.M. Lee. 1999. In vitro chemopreventive effects of plant polysaccharides (*Aloe barbadensis* Miller, *Lentinus edodes, Ganoderma lucidum* and *Coriolus versicolor*). Carcinogenesis 20:1637-1640.

Klein, A.D and Penneys N.S. 1988. *Aloe vera*. Journal of the American Academy of. Dermatology 18:714-720.

Ringo, E. and T.H. Birkbeck. 1999. Intestinal microflora of fish larvae and fry. Aquaculture Research 30:73-93.

Sahu, M.K., N.S. Swarnakumar and K. Sivakumar. 2008. Probiotics in aquaculture: importance and future perspectives. Indian Journal of Microbiology 48:299-308.

Smith, P., M.P. Hiney and O.B. Samueksen. 1994. Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Annual Review of Fish Diseases 4:273-313.

Sunitha K.S. 2012. Probiotics as growth promoter and immunostimulator in striped murrel *Channa striatus*. Ph.D thesis. Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu; Centre for Aquaculture Research and Extension (CARE), St. Xavier's College, Palayamkottai, Tamil Nadu.