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Impact of Gut Probiotics *Bacillus sp* with Minerals on Biochemical Profile of Vibriosis Infected Shrimp *Penaeus monodon* (Fabricius)

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Abstract: Biochemical profile of healthy shrimp, diseased shrimp and probiotic feeded shrimps were studied by culture in tanks for 40 days and results were compared. 20 healthy shrimps were kept as control, with out gut probiotic. 80 diseased shrimps were divided in to four groups, Group 1 kept as diseased one with out probiotic and minerals, group 2 kept as only with minerals, group 3 kept as only with probiotic, were as group 4 kept with probiotic and minerals. Protein (Group1=30.0±0.9 mg/dl, Group4=67.5±0.8mg/dl), Fat (Group1=4.0±0.3mg/dl, Group4=12.0±0.4mg/dl), Calcium (Group1=0.02±0.1 mg/dl, Group4=4.0±0.5mg/dl), Phosphorus (Group1=0.02±0.002 mg/dl, Group4=3.0±0.8mg/dl) and Fibre (Group1=0.02±0.002mg/dl, Group4=2.5±0.4mg/dl) profile clearly indicates probiotic with mineral mix helps the shrimps to recover from vibriosis.

Key words: Gut probiotics · Shrimp farm · *P monodon* · Biochemical composition · Vibriosis

INTRODUCTION

Aquaculture is a promising enterprise for economic and growing nutritional requirement of mankind. It holds much promise for meeting increasing food demands, Vibrios are among the most important bacterial pathogens of cultured shrimp responsible for a number of diseases and mortalities up to 100% have been reported due to vibriosis [1]. One of the alternatives to antimicrobials in disease control could be the use of probiotic bacteria as microbial control agents [2]. Probiotics such as the Gram positive *Bacillus* offers an alternative to antibiotic therapy for sustainable aquaculture. *Bacillus* species offer several advantages over Gram Gram negative bacteria, including longer shelf life, because they produce endospores that are tolerant to heat and desiccation and the broad spectrum activities of their secondary metabolites [3,4]. *In vitro* production of inhibitory compounds toward known pathogens for the considered species has often been used in the selection of putative probiotics [2]. *Bacillus* spp. Might produce some metabolites, for instance, antibiotic [5] or enzymes for inhibition and/or digestion [6].

In this study we have compared the Effect of Minerals, probiotics and minerals with probiotics on the biochemical biochemical profile of the diseased prawn.

MATERIALS AND METHODS

Gut Probiotic and Minerals: Commercially available Gut probiotic "Lactact" and Mineral mix "Regular-AVM" were collected from Poseidon Biotech, Chennai, India. "Lactact" has mainly *Lacto bacillus sp*, "Regular-AVM" has Amino acids, Vitamins and Minerals. 1.5g of each product has mixed with 100g of shrimp feed.

Experimental Shrimps: Morphologically and microbiologically identified 80 vibriosised and 20 healthy shrimps each 18±0.5g weighed animals were collected from culture ponds in farming area near Pattukkottai, India. Shrimps were divided into Control-Healthy, Group 1-Infected, with out Minerals and probiotics, Group 2-Infected, with Minerals, Group 3-Infected, with probiotics, Group 4-Infected, with minerals and probiotics. Shrimps were cultured for 40days in five 100 L tanks, feeded 4times daily with supplemented commercial feeds.

Biochemical Analysis: Protein, Fat, Calcium, Phosphorus and Fiber was estimated by kjeldahl method- , Babcock method, Clark Collip, Fiske and Subbarow method and Weende's method respectively [7].

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Table 1: Biochemical profile of diseased shrimp

	Days				
Parameters (mg/dl)	Initial- 0	10	20	30	40
Protein	60.0±0.4	51.0±0.7	42.0±0.5	35.0±0.6	28.0±0.6
Fat	6.0±0.3	5.6±0.4	5.0±0.5	4.4±0.4	3.8±0.3
Calcium	1.0±0.1	0.8±0.1	0.6±0.1	0.4±0.1	0.2±0.1
Phosphorus	0.5±0.1	0.3±0.1	0.1±0.01	0.05±0.01	0.02±0.01
Fiber	0.2±0.1	0.15±0.01	0.1±0.01	0.05±0.01	0.01±0.001

Table 2: Biochemical profile of only minerals feeded diseased shrimp

	Days				
Parameters(mg/dl)	Initial- 0	10	20	30	40
Protein	60.0±0.4	61.0±0.5	62.0±0.5	63.0±0.6	64.5±0.6
Fat	6.0±0.3	6.3±0.3	6.5±0.3	6.8±0.4	7.0±0.3
Calcium	1.0±0.1	1.1±0.1	1.2±0.1	1.3±0.1	1.5±0.1
Phosphorus	0.5±0.1	0.55±0.1	0.6±0.1	0.7±0.1	0.8±0.1
Fiber	0.2±0.1	0.25±0.1	0.3±0.1	0.4±0.1	0.5±0.1

Table 3: Biochemical profile of only probiotics feeded diseased shrimp

	Days				
Parameters (mg/dl)	Initial- 0	10	20	30	40
Protein	60.0±0.5	62.0±0.5	64.0±0.5	66.5±0.5	68.0±0.5
Fat	6.0±0.3	6.5±0.3	6.8±0.3	7.1±0.4	7.4±0.3
Calcium	1.0±0.1	1.5±0.1	1.8±0.1	2.1±0.1	2.5±0.1
Phosphorus	0.5±0.1	0.8±0.1	1.0±0.01	1.2±0.01	1.5±0.1
Fiber	0.2±0.1	0.3±0.1	0.5±0.1	0.8±0.1	1.0±0.1

Table 4: Biochemical profile of probiotics with minerals feeded diseased shrimp

	Days				
Parameters (mg/dl)	Initial- 0	10	20	30	40
Protein	60.0±0.5	63.5±0.5	66.0±0.5	69.5±0.5	72.0±0.5
Fat	6.0±0.3	6.5±0.2	7.0±0.2	7.5±0.2	8.0±0.2
Calcium	1.0±0.1	1.6±0.1	2.2±0.1	2.6±0.1	3.0±0.1
Phosphorus	0.5±0.1	0.8±0.1	1.2±0.1	1.7±0.1	2.0±0.1
Fiber	0.2±0.1	0.5±0.1	0.8±0.1	1.2±0.1	1.5±0.1

RESULTS

The diseased, Minerals feeded, Probiotics feeded and Probiotics with minerals feeded shrimps were analyzed for biochemical profiles for every 10days once; it shows the pattern of biochemical composition.

Group I: Group I is diseased one which has feeded no probiotic and minerals. This group shows Protein was decreased from 60.0±0.4 mg/dl to 28.0±0.6 mg/dl. Fat decreased from 6.0±0.3 mg/dl to 3.8±0.3 mg/dl. Calcium decreased from 1.0±0.1 mg/dl to 0.2±0.1 mg/dl. Phosphorus decreased from 0.5±0.1 mg/dl to 0.2±0.1 mg/dl. Fiber decreased from 0.2±0.1 mg/dl to 0.01±0.001 mg/dl. (Table 1)

Group II: Group II is diseased one which has feeded with only minerals. This group shows Protein was increased from 60.0±0.4 mg/dl to 64.5±0.6 mg/dl. Fat increased from 6.0±0.3 mg/dl to 7.0±0.3 mg/dl. Calcium increased from 1.0±0.1 mg/dl to 1.5±0.1 mg/dl. Phosphorus increased from 0.5±0.1 mg/dl to 0.8±0.1 mg/dl. Fiber increased from 0.2±0.1 mg/dl to 0.5±0.1 mg/dl. (Table 2)

Group III: Group III is diseased one which has feeded with only probiotics. This group shows Protein was increased from 60.0±0.5 mg/dl to 68.0±0.5 mg/dl. Fat increased from 6.0±0.3 mg/dl to 7.4±0.3 mg/dl. Calcium increased from 1.0±0.1 mg/dl to 2.5±0.1 mg/dl. Phosphorus increased from 0.5±0.1 mg/dl to 1.5±0.1 mg/dl. Fiber increased from 0.2±0.1 mg/dl to 1.0±0.1 mg/dl. (Table 3)

Group IV: Group IV is diseased one which has feeded with probiotics and minerals. This group shows Protein was increased from 60.0±0.4 mg/dl to 72.0±0.5 mg/dl. Fat increased from 6.0±0.3 mg/dl to 8.0±0.2 mg/dl. Calcium increased from 1.0±0.1 mg/dl to 3.0±0.1 mg/dl. Phosphorus increased from 0.5±0.1 mg/dl to 2.0±0.1 mg/dl. Fiber increased from 0.2±0.1 mg/dl to 1.5±0.1 mg/dl. (Table 4).

DISCUSSION

Shrimp is a best source for easily digestible protein. Test has shown that 80-90% of protein is assailable [8]. Biological value of prawn is 80% that is it has quality protein compared to any other animal protein [9]. The muscle of prawn is characterized by high content of protein and low level of fat in comparison with other organism [10].

Protein is essential for normal function, growth and maintenance of body tissues. Its content is considered to be an important tool for the evaluation of physiological standards. It has been suggested that the protein of prawn might vary owing to the factors such as assimilation, salinity, starvation and nutrition [11]. Protein in the total body tissues of infected animal showed a sharp decline. This may due to circular lesion, muscular opacity and atrophy of muscle in the infected shrimp. The similar observation was reported in the histological studies of *p. monodon* with red disease [12].

Decreased protein contents in the tissues may also be due to tissue destruction or necrosis which disturbs the cellular function and consequent impairment in protein synthetic machinery [13]. According to Ramnarine Ramnarine [14], the low level of protein and decreased growth rate may be due to the toxin (*V.alginolyticus*) increase the protein catabolism and subsequent increase in ammonia production which will have a negative impact on growth.

This study proved the group II diseased one's protein level decreased more than 50%, but the group (V) probiotics and minerals feeded shrimp's protein level doubled as compare with untreated diseased shrimps. Ramnarine *et al.* [15] has also proved properly probiotic feeded *P. monodon* pond gives 1900 Kg /0.5ha. With maximum growth increment of 34.70g, survival of 90.31%, achieved at the DOC 130 than irregularly probiotic feeded shrimps. Higher biomass indicates the increment of biochemical profile in tissue. This may due to increased digestive effect. Digestive effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet [16].

Lipids are extremely important in maintaining structural and physiological integrity of cellular and sub- cellular membranes. Lipids are the best source of energy producers of the body through metabolism. They provides a source of indispensable nutrients and act as carriers of certain non fat nutrients, notably the fat soluble vitamins like A, D, E and K [17]. Shrimps contain less amount of fat compared to goat meat and poultry [19]. Fat content is an important organic constituent of shrimp tissues and plays an important role in energy metabolism. Decreased level of fat was noted in group II, which is in diseased condition. This may due to stress by *V.alginolyticus*, fat is mobilized to meet the energy need and also due to lipolysis or the mitochondrial injury. The toxin produced by *V. alginolyticus* would have impaired the function of TCA cycle and fatty acid metabolism [19].The muscle among the body tissue is the main metabolic organ in prawn and it is involved in regulation of lipid metabolism by storage and release of reserves [20]. This indicates that any injury in the muscle directly affect the lipid metabolism.

This study also shows the increment of fat in group V shrimps feeded with probiotic and minerals is incredibly high. It indicates fast recovery from disease.

Calcium have important role in strengthening the skeleton and play more crucial role in metabolism, muscle activity. Calcium regulates the contraction and relaxation of muscle and regulates the passage of substance into and out of the cells. So, calcium is one of the most important nutrients for aquatic species [21]. The level of calcium is decreased when shrimp is affected by *V. alginolyticus*. This may due to the pathogenic bacteria that inhibit the absorption of calcium by intestine. It is clear from the results; the combination of probiotics and minerals is effective control the vibriosis in *P. monodon* and also increase the calcium level. This was confirmed by enhanced calcium content of group V and reduced level of calcium group II.

Phosphorus was highly utilized during production of shrimp and this seemed to be the limiting nutrient during culture [22]. This study showed that the phosphorus level was declined during infection of *V. alginolyticus*. This may be due to the inhibition of nutrient absorption by intestine. The level of phosphorus was elevated after the treatment with probiotics and minerals this renders that this mixture enhance the nutrient absorption level in intestine. *V. alginolytics* infection decreases the fibre content in diseased shrimps (group II). Probiotics and mineral mix increases the fibre level; these two results are evident by our result.

This study result revealed –that diseased shrimp (group I) shows decreased level of biochemical profile, but other groups shows increment of biochemical profile. Even though biochemical profile of (group V) shrimps feeded with probiotics and minerals was too high. So to recover from vibriosis, using Minerals alone or Probiotics alone will not be the profitable, both these two has to be mixed with feed to obtain best result.

It was concluded that Gut probiotcs with Minerals can make vibriosised shrimp to recover from it.

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