Effect of *Sargassum wightii* incorporated feed on growth and immunity development of Asian sea bass (*Lates calcarifer*)

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Abstract

Asian sea bass (*Lates calcarifer*) is a high value demanding seafood throughout the world. Bacterial infections can cause huge losses in Asian sea bass culture, among them *Aeromonas hydrophila* are ubiquitous. Inclusion of bioactive compounds present in seaweeds into fish diets can enhance the fish growth and immunity combating the bacterial diseases. This study was conducted to determine whether *Sargassum wightii* could provide immunity to bacterial infections. Low cost feeds for Asian sea bass were formulated, and growth parameters compared. A feed trial was conducted using four treatments viz. a commercial feed, a formulated feed without seaweed as control diet, formulated feeds incorporated with 20 g/kg and 40g/kg of *S. wightii.* The effect on growth performance was evaluated on Asian sea bass stocked in tanks. The stocking density was 15 advanced fingerlings/tank, and they were grown out for 40 days with triplicates. Fish survival rate and cost of formulated feed were calculated. After the end of feed trial, nine fishes from each treatment were artificially challenged with 150 ppm dose of *A.hydrophila* and observed for 7 days. There was a significant difference in weight gain in commercial feed compared to other three treatments ($P< 0.05$), whereas there was no significant difference in FCR and SGR among all experimental diets ($P >0.05$). The challenge with *A. hydrophila* showed 11.11% disease prevalence in *L. calcarifer* fed 20g/kg of seaweed incorporated feed. Highest disease prevalence with 100% was recorded in the fish that had been fed with the seaweed free formulation. Highest level of immunity enhancement was reported using the feed that contained 20g/kg seaweed. Approximately Rs. 180.00 of cost was accrued per1 kg formulated feed while commercial feed is of Rs. 250.00.

Keywords: Sea bass, Immunity, *Aeromonas hydrophila,* seaweed.

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Introduction

Sea bass (*Lates calcarifer*) is a high value seafood in demand throughout the world. Sea bass culture has been identified as a promising investment opportunity in the marine aquaculture sector in Sri Lanka due to high export demand. The bacterial infections can cause huge losses in sea bass culture. *Aeromonas hydrophila* is a common bacteria that cause Aeromonas septicemia in a variety of fish species.

Inclusion of bioactive compounds in seaweeds can enhance the immunity and growth combating the bacterial disease. This study was conducted to develop an immunity enhancing, low cost feed type for Asian sea bass against Aeromonas septicemia using *Sargassum wightii,* and to compare the growth parameters according to the given feed type.

Materials and Methods

This experiment was conducted at an indoor laboratory at the Inland Aquatic Resource and Aquaculture Division, National Aquatic Resource Research and Development Agency, Sri Lanka. A feed trial was conducted using four dietary treatments. Dietary treatments comprised of a commercial feed (control), formulated feed without seaweed and formulated feeds with 20g/kg and 40g/kg of *S. wightii* incorporated feeds in three replicates. The commercial feed is an imported feed, which used to perform the cost analysis to check whether there is a potential to develop a cost effective feed with locally available feed ingredients. The complete randomized design was used, and the effect of these test diets on the Sea bass growth performance were evaluated on fish stocked with 15 advanced fingerlings/tanks stocking density in 12 glass tanks for a period of 40 days.

The proximate composition of the feeds was established according to the AOAC methods. Crude protein was determined by the Kjeldahl procedure (AOAC 928.08). Total lipid content was determined by Soxhlet (AOAC, 1995). Moisture was quantified by oven-drying 5 g of feed at 105 °C to constant weight (AOAC 950.46).

The weight and water quality parameters were measured weekly. Mortality, tank condition and behavioral changes of fish were recorded at each feeding time. Finally the FCR (Food Conversion Ratio), SGR (Specific Growth Rate), weight gain and survival rate were calculated after 40 days.

After the feed trial nine fish from each treatment were artificially challenged with a 150 ppm dose of *A.hydrophila* and observed for 7 days. Their abnormal behavioral changes such as aggregations, activeness, etc. disease symptoms if any and survival rate were recorded daily. Pure culture of *A. hydrophila* was obtained from NARA microbiology laboratory.

Statistical analyses were performed using Minitab 16 software package. One way ANOVA was used to test differences between the treatments at 95% confidence level.

Results and Discussion

According to proximate composition the highest protein content (45.48%) was recorded in diet 1 which is the commercial feed. The protein content of diet 2 (without seaweeds), diet 3 (20 g/kg of seaweeds) and diet 4 (40 g/kg of seaweeds) was 35.67%, 37.66% and 36.7% respectively. O'Keefe and Newman (2011) reported that the adult Sea bass requires a ration containing 35 to 45 proteins. The experimental feeds contain lower protein amounts compared to the commercial feed. It is better to develop a feed with local ingredients with high protein levels to have better result than this.

Table 1: Growth performance of Asian sea bass according to the feed type

Parameter	T1 (Mean±SEM)	T2 (Mean±SEM)	T3 (Mean±SEM)	T4 (Mean±SEM)
Initial weight(g)	2.60±0.03	2.37±0.08	2.26±0.10	2.02±0.08
Final weight (g)	6.77 ± 0.77^{a}	5.04 ± 0.45^{ab}	4.17 ± 0.25^{b}	4.23 ± 0.55^{b}
SGR	2.35 ± 0.24^{a}	1.86 ± 0.21^{a}	1.52 ± 0.087^{a}	1.80 ± 0.22^{a}
FCR	1.27 ± 0.10^{a}	2.17 ± 0.26^{b}	2.02 ± 0.059^{b}	2.21 ± 0.50^{b}

SEM – Standard Error of Mean

Values indicated by different superscript in each row are significantly different from each other ($p<0.05$). However, the weight gain in treatment 1 was significantly higher compare to other three treatments ($P< 0.05$). This might be due to the higher initial weight of the sample. There was no effect of seaweeds on the growth of fish tested. Compare to the diet 3 and 4, the weight gain was high with the diet 2. This conclusively represents that the adding of seaweed has not affected to the weight gain. The commercial feed contained higher protein content than other three diets tested. Moreover, other prepared feeds might be having a various impurities in ingredients which may be led to lower the protein content therein. In generally the growth of fish may differ with their age, size, metabolic condition, environmental condition, physiological characters and the quality of the feed. Although, same aged and same sized fish were selected for the study, one or more above factors cloud also be influenced their growth.

The mean SGR for treatment 1, treatment 2, treatment 3 and treatment 4 were not significantly different from each ($P >0.05$). SGR was expected to be around 1.5% in short periods (Hossu*et.al*, 2005). Naturally, the SGR is increased with increasing of weight gain. Accordingly the higher SGR (Table 1) should be in treatment 1 where it

has resulted higher weight gain. Feed conversion ratio is one of the most important criteria in this kind of studies. Since we have used only three replicates for each treatment, and also due to high variability of the samples, a significant difference between the treatments has not been evident. Nevertheless the feeds tested gave reduced SGR as well as poor FCR compared to the control feed tested.

There was no significant difference ($P>0.05$) in survival rate of Sea bass fed with different feeds tested. The highest survival rate was recorded in treatment 1 (97.78±2.22) and treatment 3 (97.78±2.22) followed by treatment 2(86.70±10.2) and treatment 4 (91.11±5.88), respectively. It revealed that sea weed has not affected to the survival rate of fish in all feeds types tested. However, all the treatments have shown a good survival rate. The sea bass is commonly farmed in 10-31ppt of salinity, 26-32^0 C of temperature, 7.5-8.3 of pH and 4-8 mg/L of dissolved oxygen (FAO, 1988). During the present study period, salinity was 17.6 -18 ppt, temperature was 28.2 - 28.55^{0}C, pH was 6.68- 6.78 and dissolved Oxygen was varied from 4.82 -5.54 mg/L. The maintenance of all these parameters at their optimum levels may have contributed to be reported a high survival rate during the study period.

The occurrence of disease was confirmed by the visual observation of clinical signs such as darkening of body color, loss of appetite, red eyes, swollen eyes or white spots in eyes and behavioral changes such as unbalanced condition, lethargic condition and aggregation into one corner. The most dominant clinical sign was a darkening of the body color. It was the foremost character for identifying the disease condition of this study. Other than that gradually they have lost their appetite and all infected fishes showed red, swollen and white spots in eyes. Apart from these clinical signs, behavioral changes in Sea bass such as unbalanced condition and aggregation to a corner were also observed in the Sea bass infected with *A. hydrophila.*

Percentage of disease prevalence for *Aeromonas septicemia* in four treatment groups, at the end of the artificial challenge study with *A. hydrophila*is shown figure 1.The lowest disease prevalence was reported in treatment 3 which fed with 20 g/kg of seaweed incorporated feed. The incorporation of seaweed into the feed may enhance the immunity of Sea bass fish. The formulated feed which was incorporated with 40g/kg of

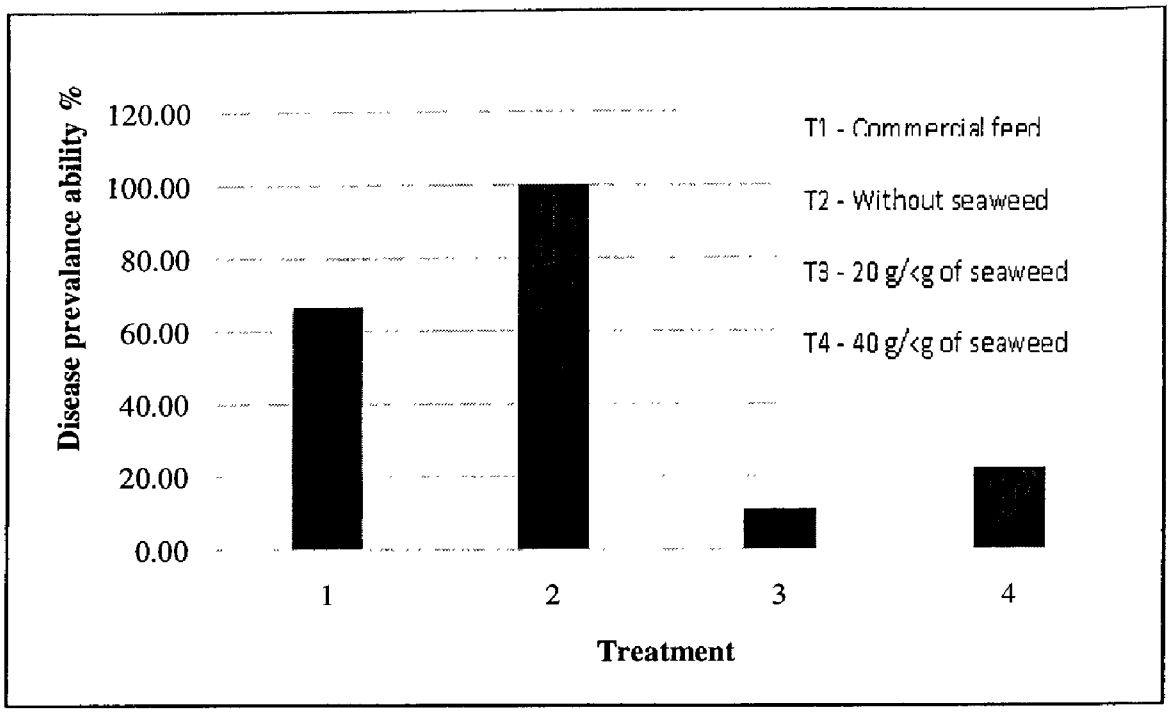

Figure 1: Comparison of disease prevalence in four different feed types tested.

seaweeds also having comparatively lower disease prevalence compared to the other two treatments. However, it was revealed that sea weeds may have potential to enhance the immunity in Sea bass fish. According to Francisca et al. (2012), the brown alga has characterized the properties of β-glucan which is a naturally occurring non-specific immunostimulants. An immunostimulant that enhances the innate immune response by interacting with cells of the immune system. The immunostimulants are promising dietary supplement to potentially aid in disease control of several organisms including marine fish and increase disease resistance by regulating host defense mechanisms against opportunistic microorganisms in the environment (Galindo and Hosokawa, 2004).

It was estimated that approximately 180.00 LKR cost aspired for the formulated feed. In the case of commercial feed it was around 250.00 LKR. However, the experimental feed gained comparatively low cost when compared to the commercial feed which promises the productivity, health and other related benefits. Identification and utilization of locally available ingredients to develop both immunity and growth enhancing low cost feed type for Asian Sea bass culture will more profitable to the poor fish farmers in Sri Lanka.

Conclusion

The present study revealed the effect of *S. wightii* incorporated feed on immunity development of Asia Sea bass. The highest level of immunity enhancement was reported with 20g/kg seaweeds incorporated feed. Since sea weed incorporated feed development is cost effective which can be developed with locally available feed ingredients, this feed will be useful in developing Sea bass culture in Sri Lanka. Since this study is not totally support to get a better conclusion for growth performances of Sea bass further research are needed to be conducted with more replicates with lowering the variability.

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