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Growth and Immune Responses of *Labeo rohita* Supplemented with Combination of Fucoidan Rich Seaweed Extract and Probiotic

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ABSTRACT

A 60 d feeding trial was conducted to study the combined effect of fucoidan rich seaweed extract (FRSE) from *Sargassum wightii* and probiotic (*Bacillus subtitlis*) on growth and immune parameters of *Labeo rohita* fingerlings. Two hundred and ten fingerlings of uniform size group were distributed into seven experimental groups in triplicates. Each groups were fed to satiation with iso-nitrogenous (35% CP) and iso-caloric (396.3 kcal/100g) purified diets containing 0% FRSE and no probiotic (control), 2% FRSE (T1), 10^8 CFU g^{-1} probiotic (T2), 1% FRSE + 10^5 CFU g^{-1} probiotic (T3), 2% FRSE + 10^5 CFU g^{-1} probiotic (T4), 1% FRSE + 10^8 CFU g^{-1} probiotic (T5) and 2% FRSE + 10^8 CFU g^{-1} probiotic (T6). Higher weight gain percentage, specific growth rate, protein efficiency ratio and lower feed conversion ratio were recorded in T3, T5 and T6 groups. After feeding trial, the experimental fish were challenged with *Aeromonas hydrophila* and the best relative survival rate was observed in T6 group followed by T4 whereas, albumin/globulin (A/G) ratio and haemoglobin content exhibited decreasing trend. SOD and catalase activity in liver and muscle were found significantly lower in the groups fed with fucoidan. Hence, both innate immunity and growth parameters can be enhanced using the combination of FRSE and probiotic in the diet of *L. rohita*.

Key Words: *A. hydrophila*, Fucoidan, Growth parameters, Innate immunity, Probiotic.

INTRODUCTION

Aquaculture is expanding in new directions, with intensification and diversification to meet the constant increase in consumer demand for the fish and fishery products. Traditional use of antibiotics and other chemotherapeutics in fish culture has been criticized due to potential development of antibiotic-resistant bacteria, accumulation of antibiotics in the environment and residual accumulation in fish (Merrifield *et al*, 2010). Researchers have intensified efforts to identify and develop safe dietary supplements and additives that enhance growth and immune responses of cultured fish (Ji *et al*, 2007; Shim *et al*, 2009) since the Regulation 1831/2003/EC on ban on the use of all sub-therapeutic antibiotics came in to action.

Increased attention is being paid to the use of immune stimulants which facilitates functions of phagocytic cells by increasing their bactericidal activity, stimulate the natural killer cells, complement system, lysozyme activity and antibody responses in fish and shellfish for disease control measures in aquaculture (Harikrishnan *et al*, 2011). *Bacillus subtilis* is known as a potential probiotic in aquaculture (Olmos and Paniagua-Michel, 2014) and extensively studied for its immune stimulatory effects and pathogen control in aquaculture (Mohapatra *et al*, 2013). Oral administration of *B. subtilis* controlled occurrence of infection in *Aeromonas sp*. (Newaj-Fyzul *et al*, 2007) and *Yersinia ruckeri* (Raida *et al*, 2003) challenged rainbow trout, *A. hydrophila* and

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Pseudomonas fluorescens challenged Nile tilapia (Aly *et al*, 2008) and *Vibrio sp.* challenged *Penaeus monodon* (Vaseeharan and Ramasamy, 2003).

Fucoidan is a polysaccharide which contains large amounts of l-fucose and sulfate, composed with minor proportions of xylose, galactose, mannose and glucuronic acid (Duarte and Prairie, 2001). Phaeophycophyta (Brown seaweeds) are known to produce fucoidans (Painter, 1983). Immune potentiating activity of fucoidan has been extensively investigated in fishes such as Nile tilapia, *Oreochromis niloticus* (Isnansetyo *et al*, 2016), in sutchi cat fish, *Pangasianodon hypophthalmus* (Prabu *et al*, 2016) and in *Labeo rohita* (Gora *et al*, 2018). Fucoidan is known to induce immune response in crustaceans as well (Sinurat *et al*, 2016; Canciyal *et al*, 2016). Growth stimulating effect of fucoidan was found in juvenile red sea bream, *Pagrus major* at the dietary inclusion level of 0.2-0.4% (Sony *et al*, 2018) while no effect was reported in *L. rohita* (Gora *et al*, 2018).

The immunostimulating modulation of fucoidan has been found to be effective at 2 per cent level of inclusion in the diet of *L. rohita* (Gora *et al*, 2018). The economic feasibility of this fucoidan depends upon its minimum inclusion level as maximum 5 per cent recovery of fucoidan has been reported from different seaweeds (Flórez-Fernández *et al*, 2017; Prabhu *et al*, 2016; Gora *et al*, 2018). Moreover, the synergistic effect of fucoidan with any other additives which can stimulate immunity at lower dose along with augmentation of growth is a researchable issue. Hence, this study aimed to investigate the interaction of dietary fucoidan and probiotic on growth and non-specific immune responses of cultured fish.

MATERIALS AND METHODS

Seaweed collection, fucoidan extraction and quantification

Sargassum wightii, a brown seaweed was collected from Mandapam, Ramanathapuram district of Tamil Nadu, India with the help of local fishermen. The speceis identity was confirmed using satndard identification keys (Vijayabaskar and Shiyamala, 2011). Freshly collected, matured and healthy brown seaweeds were thoroughly washed with water, shade dried for two days and ground into fine powder. Extraction of fucoidan was done using the alcohol-water method (Usui *et al*, 1980) and the crude fucoidan was purified by EDTA (1mM for 10 minutes) activated dialysis membrane. The dialysed sample was used for quantification in terms of L-fucose by following the method of Dubois *et al* (1956).

Estimation of DPPH scavenging

DPPH scavenging activity of crude fucoidan extract was measured by modified method of Brand-Williams *et al* (1995). The deep purple chromogen of DPPH (2,2-diphenyl-1-picrylhydrozyl) radical is reduced by antioxidant/reducing compound to the corresponding pale-yellow hydrazine. Methanolic DPPH (2 ml of 0.06 M) was added to the gradient concentrations of fucoidan sample and mixed well. The optical density was measured at 517 nm against the reagent blank after incubating the mixture in dark for 30 minutes. The control containing no fucoidan extract was taken as reagent blank.

Bacterial strain and culture

The pure bacterial isolate of *B. subtilis* (MTCC No. 10407) was received from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India as lyophilized cells. The bacteria were inoculated into conical flask containing nutrient broth (Hi-Media) and kept in BOD incubator for 24 hr at 30 ^{o}C. After which a loopful of bacterial culture was streaked on Nutrient agar (Hi-Media) plate and incubated for 24 hr at 30 ^{o}C. Culture was confirmed as pure isolates of respective culture by performing the essential biochemical tests and was used for mass culture for subsequent use in the experiment.

Quantification of inoculums

To determine the concentrations of the bacterial inoculums to be added into the feed for the experiment, bacteria was streaked on nutrient

broth and incubated for 24 h at 30 °C. One freshly grown colony was picked and transferred into 50 ml of nutrient broth, and incubated under the same conditions for 4 hr. A second transfer was carried out into 100 ml, under same conditions. Then optical density (OD) at 600 nm was recorded, simultaneously serial dilutions were performed for each hour. The dilutions were plated onto the nutrient agar by spread plate technique. After 12 hr of incubation at 28 °C colonies were counted. The data were related in graphs, obtaining the relationship CFU vs. OD_{600} vs. time.

Experimental animal

Juveniles of rohu, *Labeo rohita* were procured from Prem Fisheries Consultancy, Ankleshwar, Gujarat. The juveniles were transferred to 400 L FRP tanks with vigorous aeration for acclimation. In order to ameliorate the handling stress, the fish were given 0.2% salt treatment for 2 min in the next morning. The stock was acclimatized with vigorous aeration and fed with 35 percent crude protein diet.

Experimental design and set-up

The experiment was conducted over a period of 60 d at the Wet Laboratory of ICAR-Central Institute of Fisheries Education in 21 plastic rectangular tubs (80×57×42 cm, 100 L capacity) covered with plastic lids. The tubs were initially washed with potassium permanganate solution (5 mg/ml) and were left overnight. The tanks were flushed out on the next day and then thoroughly washed with water. About 210 fingerlings were randomly distributed in the five distinct experimental groups with each of three replicates. The experimental groups were designated as C (control), T1 (2% Fucoidan), T2 (Probiotic high dose, *Bacillus subtilis* 10^8 CFU g^{-1}), T3 (1% Fucoidan + low dose probiotic, 10^5 CFU/g), T4 (2% Fucoidan + low dose probiotic, 10^5 CFU/g), T5 (1% Fucoidan + high dose probiotic, 10^8 CFU/g) and T6 (2% Fucoidan + high dose probiotic, 10^8 CFU/g).

Formulation and preparation of experimental diets

Purified ingredients were used to prepare the diets. The ingredients were brought from HiMedia Chemical Ltd. Seven diets of same composition except the cellulose, fucoidan and probiotic were prepared in the Fish Nutrition Laboratory of ICAR-CIFE. The cellulose powder was used as filler to make up the volume as equal quantity in all the diets. Composition of diets is given in Table 1. Ingredients except vitamin mineral mix, additives and oils were mixed well and cooked in steam while the heat labile ingredients were mixed before pelletizing.

Sampling

The body weight gain of fishes was recorded on fortnightly basis. Muscle, liver, gill and intestine tissues of the fishes were removed aesthetically, weighed and were homogenized in chilled sucrose solution (0.25 M) using tissue homogenizer on ice. The homogenate was centrifuged at 5000 rpm for 10 min at 4 °C. The supernatant was stored at -20 °C until use. Blood was collected from caudal vein using EDTA treated syringe and transferred to EDTA vials. Blood was collected using untreated syringe for serum and allowed to clot at room temperature for 10- 20 min. The serum was collected after spinning the vials at 3000 rpm for 10 min.

Growth parameters

The parameters were calculated as follows;

Percent weight gain = $(W_t - W_0) \times 100 / W_0$,

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100$ / number of days,

Feed Conversion Ratio (FCR) = feed given- weight gain,

Protein Efficiency Ratio (PER) = Weight gain/ Protein fed

Oxidative enzymes analysis

Superoxide dismutase (SOD) was assayed according to the method described by Misra

Table 1. Composition of the experimental diets (% DM basis).

Ingredient	Control	T1	T2	T3	T4	T5	T6
Casein*	33	33	33	33	33	33	33
Gelatin*	7.25	7.25	7.25	7.25	7.25	7.25	7.25
Dextrin*	16.75	16.75	16.75	16.75	16.75	16.75	16.75
Starch soluble*	19.5	19.5	19.5	19.5	19.5	19.5	19.5
Cellulose*	11	9	11	10	9	10	9
Cod liver oil[#]	4	4	4	4	4	4	4
Sunflower oil[#]	4	4	4	4	4	4	4
Vit.-min. premix[c]	2	2	2	2	2	2	2
CMC[a]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
BHT[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline chloride[*]	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Fucoidan	0	2	0	1	2	1	2
Probiotic(CFU g^{-1})	Nil	Nil	10^8	10^5	10^8	10^5	10^8
Total	100	100	100	100	100	100	100

[a]Carboxymethyl cellulose, [b]Butylated Hydroxyanisol, [c]Brand: Virbac

[*] Purified ingredient procured from HiMedia Laboratories, India

[#] Oils procured from local market

and Fridovich (1972) based on the oxidation of epinephrine–adrenochrome transition by the enzyme. Catalase activity was assayed according to the method described by TaKa Ra *et al* (1960) using H_2O_2 solution.

Total serum protein, Albumin, Globulin and A/G

Serum protein was estimated by Biuret method (Reinhold, 1953) using Innoline™ Total Protein Plus kit. Albumin was estimated by Bromocresol green binding method (Doumas and Biggs, 1972). Globulin was calculated by subtracting albumin values from total plasma protein. A/G ratio was calculated by dividing albumin values by globulin values

Haematological parameters

The hemoglobin level of blood was analysed following the Cyanmethemoglobin method using Drabkins Fluid (Qualigens). Total erythrocyte count (TEC) was analysed using Neubauer's counting chamber of haemocytometer and observing under 40X magnification of light microscope. Total leucocyte count (TLC) was also analysed the same way. Packed cell volume (PCV) / Haematocrit value was determined by drawing non-clotted blood by capillary action into microhaematocrit tubes. One end of the tubes was sealed with synthetic sealant. The sealed tube was centrifuged in a microhaemotocrit centrifuge for 5 min at 10,500 rpm. The PCV measured using microhaemotocrit reader and expressed as percentage.

Challenge study

After immunomodulation trial through feed over 45 d duration, the fish in various experimental groups were challenged with the pathogenic isolates of *A. hydrophila.* For this, the pathogenic isolates of *A. hydrophila* were grown on nutrient agar for 24 hr at 30 °C in a BOD incubator. The cells were harvested and washed thrice in sterile

Table 2. L-fucose content and yield of crude fucoidan from *S. wightii.*

L-fucose content (%)	Yield of crude fucoidan (g/100g dried seaweed powder)
28.59±0.76	5.03±0.17

Table 3.Antioxidant assays of *S. wightii* extracts of different concentrations (DPPH, % inhibition).

Concentration (mg/ml)	0.25	0.5	1	2	4	6	8	10	12
DPPH (% inhibition)	9.81 ±1.48	13.01±0.93	16.92±0.62	21.39±0.31	36.74±1.86	42.00±0.98	50.11±0.77	59.35±0.43	60.63± 0.63

(Data expressed as Mean ± SE, n=3)

PBS and then resuspended in PBS at concentration of 10^5 cells ml^{-1}. The fishes in each experimental group were injected with 0.2 ml of this suspension intra-peritoneally. The cumulative mortality (%) patterns were observed in the challenged fishes up to a week along with the changes in behaviour and morphology. The cause of death was ascertained by re-isolating the infecting organism from dead fish liver.

Statistical analysis

The data were statistically analyzed by using statistical package SPSS version 22. The data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences between the treatments. Paired t-test used to determine the significant difference between the mean of pre- and post-challenge treatments. Comparisons were made at the 5% probability level.

RESULTS AND DISCUSSION

Yield of crude fucoidan

The dialysed fucoidan extracts were quantified by measuring the L-fucose content, which was multiplied by a factor of 1.75 to get the total fucoidan content. The L-fucose content of the concentrated and dialysed extract of species *S. wightii* is given in Table 2.

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging

The free radical scavenging capacity of the crude fucoidan extracts with different concentrations was tested using the stable free radical, DPPH. The ability of each concentration of fucoidan to scavenge DPPH radical is represented as percentage inhibition (Table 3). The crude extract exhibited varying degrees of scavenging capacity depending on the concentration.

Growth parameters

The body weight gain percentage of control and T1 were not differed significantly ($P \geq 0.05$). Significantly higher weight gain ($P \leq 0.05$) was recorded in T3, T5 and T6 groups. SGR and PER were exhibited similar trend. Similarly, lowest FCR was observed in T5 (2.22±0.12) followed by T3 and T6. The FCR of treated groups were significantly lower from the control group (Table 4).

Oxidative enzyme activities

The SOD activity in the liver differed ($P \leq 0.05$) among various groups both in the pre- and post-challenge periods. Significantly highest ($P \leq 0.05$) SOD activity in the liver was recorded in control (37.64±1.26 and 48.39±1.42) group and lowest activity was observed in T1, T4, T5 and T6 in both pre- and post-challenge conditions. In post challenge study, SOD activity was significantly lower ($P \leq 0.05$) in all the treated groups from control and T2. The pre- and post-challenge catalase activity of the different experimental group is shown in Table 5. In the liver, the catalase activity followed the similar trend and the activity of the control

Table 4. Growth parameters of the different experimental groups.

Treatments	Wt. Gain %	SGR	PER	FCR
C	$77.74^{a}\pm3.84$	$0.96^{a}\pm0.04$	$0.95^{a}\pm0.04$	$2.95^{d}\pm0.12$
T1	$91.25^{ab}\pm3.92$	$1.08^{ab}\pm0.03$	$1.08^{b}\pm0.03$	$2.62^{c}\pm0.07$
T2	$95.29^{bc}\pm4.27$	$1.11^{bc}\pm0.04$	$1.1^{bc}\pm0.04$	$2.57^{bc}\pm0.10$
T3	$112.92^{de}\pm5.93$	$1.26^{de}\pm0.05$	$1.24^{de}\pm0.05$	$2.29^{ab}\pm0.08$
T4	$98.10^{bcd}\pm3.19$	$1.14^{bcd}\pm0.06$	$1.11^{bcd}\pm0.02$	$2.53^{bc}\pm0.05$
T5	$119.27^{e}\pm7.93$	$1.31^{e}\pm0.06$	$1.28^{e}\pm0.06$	$2.22^{a}\pm0.12$
T6	$110.46^{cde}\pm5.38$	$1.24^{cde}\pm0.04$	$1.23^{cde}\pm0.03$	$2.31^{ab}\pm0.07$

group was significantly higher ($P\leq0.05$) than other treatment groups in the both pre- and post-challenge conditions. In the post challenge period, the activity of each treatment group increased compared to their pre-challenge counterparts (Table 5).

Haematological parameters

Treatment groups fed on fucoidan exhibited higher values of different haematological parameters in both pre- and post- challenge conditions. Haemoglobin percentage and haematocrit value found to be higher in groups T6, T5 and T4, though there was no significant difference ($P\geq0.05$) among these groups. Total erythrocyte count was similar among the treatment groups. Mainly, the values of total leucocyte count differed significantly among the experimental groups in both pre- and post-challenge conditions. All the treatment groups except T2 were found to have significantly higher ($P\leq0.05$) leucocyte count in blood; among which value of T6 group was highest followed by T4 in both pre- and post-challenge conditions.

The pre-challenge and post-challenge total leucocyte count (TLC) of the different treatment groups are given in Table 6. In both the periods, highest TLC obtained in T6 followed by T4. No difference in TLC count was observed between

Table 5. Pre- and post-challenge SOD activity in liver and gill of different experimental groups.

Treatment	SOD (Liver)		Catalase (Liver)	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
C	$31.05^{bA}\pm2.31$	$3.91^{bA}\pm0.55$	$3.91^{bA}\pm0.55$	$48.39^{cB}\pm1.42$
T1	$17.05^{aA}\pm2.12$	$1.35^{aA}\pm0.34$	$1.35^{aA}\pm0.34$	$25.52^{aB}\pm0.27$
T2	$29.08^{bA}\pm1.89$	$3.60^{bA}\pm0.68$	$3.60^{bA}\pm0.68$	$34.85^{bB}\pm1.10$
T3	$25.58^{bA}\pm3.76$	$1.82^{aA}\pm0.19$	$1.82^{aA}\pm0.19$	$32.80^{bB}\pm2.31$
T4	$14.29^{aA}\pm0.54$	$1.29^{aA}\pm0.24$	$1.29^{aA}\pm0.24$	$24.58^{aB}\pm1.78$
T5	$15.48^{aA}\pm0.51$	$1.52^{aA}\pm0.28$	$1.52^{aA}\pm0.28$	$31.73^{bB}\pm2.09$
T6	$16.64^{aA}\pm3.52$	$1.24^{aA}\pm0.40$	$1.24^{aA}\pm0.40$	$23.13^{aB}\pm0.69$

Superscripts in lower case implies the difference between treatment effects vertically in a column and upper case implies the difference between pre- and post-challenge horizontally for a parameter. Data expressed as Mean ± SE

Table 6. Pre- and post-challenge blood parameters of different experimental groups.

Treatment	Hb (g%)		TEC (10^6/mm^3)		TLC (10^3/mm^3)		HCT (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
C	$9.17^{aA}\pm0.07$	$8.20^{abB}\pm0.12$	$2.50^{aA}\pm0.12$	$2.20^{bB}\pm0.06$	$13.33^{aA}\pm0.61$	$15.87^{aA}\pm0.78$	$40.00^{aA}\pm0.58$	$36.00^{abA}\pm0.58$
T1	$9.30^{abA}\pm0.12$	$8.23^{abA}\pm0.18$	$2.60^{aA}\pm0.06$	$2.13^{abA}\pm0.09$	$18.60^{bA}\pm0.49$	$21.63^{bA}\pm0.41$	$42.33^{abA}\pm0.88$	$37.33^{abA}\pm0.88$
T2	$9.33^{abA}\pm0.12$	$7.90^{aB}\pm0.12$	$2.57^{aA}\pm0.09$	$2.00^{aA}\pm0.06$	$12.30^{aA}\pm0.83$	$14.33^{aA}\pm0.39$	$43.00^{abA}\pm1.53$	$35.33^{abA}\pm0.88$
T3	$9.47^{abcA}\pm0.12$	$8.23^{abB}\pm0.09$	$2.63^{aA}\pm0.09$	$2.00^{aB}\pm0.06$	$17.23^{bA}\pm1.26$	$19.6^{aA}\pm0.32$	$43.00^{abA}\pm1.15$	$35.00^{aB}\pm0.58$
T4	$9.57^{bcA}\pm0.07$	$8.40^{bcB}\pm0.12$	$2.77^{aA}\pm0.07$	$2.17^{abB}\pm0.03$	$21.83^{cA}\pm0.69$	$25.5^{cB}\pm0.32$	$43.33^{bA}\pm0.88$	$36.00^{abB}\pm1.15$
T5	$9.73^{cA}\pm0.09$	$8.57^{bcB}\pm0.07$	$2.67^{aA}\pm0.07$	$2.10^{abB}\pm0.06$	$19.77^{bcA}\pm0.59$	$21.3^{aA}\pm0.91$	$44.67^{bA}\pm0.67$	$36.67^{abB}\pm0.88$
T6	$9.67^{cA}\pm0.07$	$8.67^{cB}\pm0.07$	$2.77^{aA}\pm0.07$	$2.13^{bcB}\pm0.03$	$25.27^{dA}\pm1.43$	$28.27^{dB}\pm1.48$	$45.00^{bA}\pm0.58$	$38.00^{bB}\pm0.58$

Superscripts in lower case implies the difference between treatment effects vertically in a column and upper case implies the difference between pre- and post-challenge horizontally for a parameter. Data expressed as Mean ± SE.

control and T2, while other treatment groups significantly different (P≤0.05) from control. Similar pattern in TLC count was obtained after challenge study as well. TEC and Hb count were not significantly differs from each in both in pre- and post-challenge study.

Serological parameters

In this experiment, significant difference (P≤0.05) was observed in total protein content of the serum of the treatment groups compared to the control. Globulin content was found to be significantly highest (P≤0.05) in T4 group in both pre- and post-challenge conditions. Serum albumin to globulin ratio was determined for both the pre-challenge and post-challenge periods (Table 7). In both the periods, the lowest A/G ratio was observed in the T4 and T6 groups. Post-challenge values were consistently higher than their pre-challenge counterpart.

Survival rate

The relative survival and survival rate of *L. rohita* when challenged with *A. hydrophilla* in different experimental groups are presented in Fig. 1. The highest survival rate was recorded in T6 group (70%). The highest relative survival rate was also recorded in the T6 group (57%) when challenged with *A. hydrophilla.*

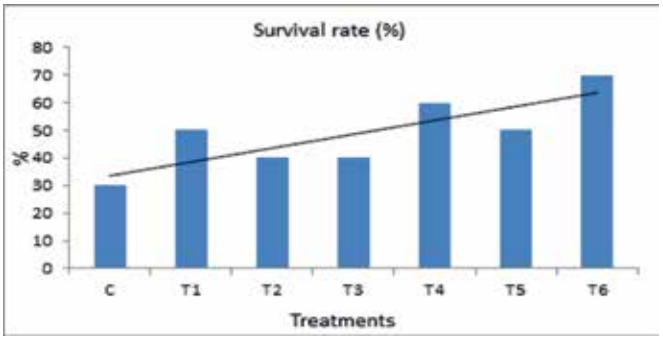

Fig 1. Survival rate (%) of *L. rohita* fingerlings of different experimental groups challenged with *A. hydrophilla*

Table 7. Pre- and post-challenge serum protein, albumin, globulin and A:G ratio of the different experimental groups

Treatment	Pre-challenge				Post-challenge			
	Total protein	Albumin	Globulin	A/G ratio	Total protein	Albumin	Globulin	A/G ratio
C	3.13^{a}±0.03	1.45±0.03	1.73^{a}±0.01	0.84^{e}±0.02	2.95^{a}±0.04	1.41^{bc}±0.02	1.54^{a}±0.03	0.92^{e}±0.01
T1	4.16^{c}±0.05	1.73±0.02	2.43^{c}±0.03	0.71^{d}±0.01	3.71^{c}±0.08	1.64^{de}±0.04	2.07^{c}±0.05	0.79^{cd}±0.01
T2	3.71^{b}±0.11	1.57±0.09	2.13^{b}±0.02	0.74^{d}±0.04	3.39^{b}±0.02	1.53^{cd}±0.02	1.86^{b}±0.02	0.82^{c}±0.02
T3	3.92^{bc}±0.12	1.44±0.09	2.48^{c}±0.04	0.58^{bc}±0.03	3.78^{c}±0.06	1.58^{cde}±0.06	2.19^{c}±0.01	0.72^{bc}±0.03
T4	5.33^{e}±0.19	1.73±0.13	3.59^{e}±0.08	0.48^{a}±0.03	4.87^{e}±0.05	1.76^{e}±0.06	3.11^{e}±0.10	0.57^{a}±0.04
T5	4.15^{c}±0.22	1.56±0.13	2.59^{c}±0.11	0.60^{c}±0.04	3.39^{b}±0.14	1.33^{b}±0.11	2.06^{c}±0.03	0.64^{ab}±0.05
T6	4.67^{d}±0.11	1.57±0.05	3.09^{d}±0.06	0.51^{ab}±0.01	4.29^{d}±0.06	1.54±0.05	2.75^{d}±0.04	0.56^{a}±0.02

Superscripts in lower case implies the difference between treatment effects vertically in a column. Data expressed as Mean ± SE.

Growth and immune response

Studies on the effect of fucoidan on growth performance are limited. In the present study, growth responses such as weight gain percentage, SGR, PER and FCR of 2% fucoidan rich seaweed extract from *S. wightii* fed groups were not significantly different from control. Gora *et al* (2018) and Prabu *et al* (2016) observed similar effect of fucoidan on growth performance in *L. rohita* and *P. hypophthalamus,* respectively. While, *B. subtilis* (at 10^8 CFU g^{-1}) treatment alone had significantly improved growth response from control, though which were not different from fucoidan alone fed groups. One per cent fucoidan fed groups exhibited better growth response than 2 per cent fed groups in both low and high dose probiotic fed groups. Highest growth was obtained in T5 group (1% fucoidan and *B. subtilis* at 10^8 CFU g^1), which was not significantly different from T3 (1% fucoidan and *B. subtilis* at 10^5 CFU g^1). Traifalgar *et al* (2010) and Tuller *et al* (2012) reported that inclusion of 1 per cent fucoidan rich extract from *Undaria pinnafitida*is is sufficient to enhance the growth performance of shrimp and juvenile sea bass, respectively.

Superoxide dismutase catalyses the dissociation of superoxide into oxygen and hydrogen peroxide. Catalase converts this toxic hydrogen peroxide into oxygen and water. As such, they are important antioxidant enzymes found nearly in all cells exposed to oxygen. In the present study, the SOD and CAT activity was found to decrease significantly both in gill and liver in all the treatment groups except T2 (and T3) compared to the control. It might be due to the presence of phenolic compounds and sulphur content of fucoidan (Jin *et al*, 2014). Thus fucoidan can complement the antioxidant enzyme systems of the animal and hence the activity of the enzymes will be lower. As T2 group fed on fucoidan free diet no significant decrease in the SOD or CAT activity was noticed. In post-challenge condition, the activity of both of these enzymes increased for all the experimental groups. But control group showed the highest activity of both these enzymes than other treatment groups.

In the present study, the highest relative rate of survival was observed in T6 group followed by T4 group. Both these groups were fed with diet containing both fucoidan (at 2% level) and probiotic. All the experimental groups showed higher survival rate compared to the control. This might be due to the triggering of the non-specific immune system of the fish by the synergistic effect of fucoidan extract and probiotic bacteria.

CONCLUSION

Present result indicated that the combined effect of FRSE and probiotic at lower dose was beneficial to the cultured species both in terms of growth and immune parameters. This is the first study of its kind where growth aspect is taken care along with immunostimulating capacity of fucoidan paving the way for the development of nutraceuticals for the aquaculture. Hence, further studies with different species are required to fully understand the role and effects on fish of this master combination.

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