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Role of Immunostimulants in Aquaculture: Suggestive Experimentation on the Immuno-Nitrogenous Effect of Dried Ginger (*Zingiber officinale*) and Teak Leaves (*Tectona grandis*) in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT: Fisheries and aquaculture industry stay unaltered as an unavoidable wellspring of food, income, nourishment and livelihood for a huge number of individuals all throughout the world. It has been viewed as one of the quickest developing agricultural business areas of the world. In this unique situation, the present interesting experimentation will assess the capability of dried ginger and teak leaf in increasing the general wellbeing and resistance of Nile tilapia. In this experiment the segments of the fundamental control diet comprise of fishmeal (26%), com flour (21%), soybean supper (24%), wheat flour (6%), rice grain (16%), fish oil (0.5%), cellulose (5%) and a premix (1.5%). Five trial diets will be set up with 2.5 g/Kg, 5 g/Kg, 7.5 g/Kg, 10 g/Kg and 12.5 g/Kg of dried ginger and teak leaf powder. Following 30 days taking care of preliminary, five fish from each tank must be haphazardly chosen, and tested with 0.1ml of saline arrangement of *A. hydrophila*. Challenge study goes on for 15 days and every day, mortality will denoted. Changes in the count of various blood contents were estimated and tabled in the study. It was clear to note that the level of Ca, Na and K and immunoglobulin (IgM Value) in plasma were increased dramatically with the increasing levels of ginger and teak leaf. The highest cumulative survival was accomplished in fish fed with the addition of 2.5 g *Z. officinale* and *Tectona grandis* extract per kg of feed, trailed by fish in the treatment of 1.25 g/kg of feed. It can be concluded that supplementation of ginger and teak leaf extract as an alternative to therapeutic agents and antibiotics had additive benefit in the immuno- resistance and growth performance of fish compared with the control.

Abbreviations: ANOVA, Analysis of Variance; Ca, Calcium; ds RNA, Double Stranded Ribonucleic Acid; EDTA, Ethylene Diamine Tetra Acetic Acid; FAO, Food and Agriculture Organization; FCR, Food Conversion Ratio; GH, Growth Hormone; HCT, Hematocrit; IgM, Immunoglobulin; K, Potassium; LPS, Lipopolysaccharide; MCV, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; MPEDA, Marine Product Export Development Authority; Na, Sodium; PCV, Packed Cell Volume; RBC, Red Blood Cell; RNA, Ribonucleic Acid; RT-qPCR, Quantitative Reverse Transcription Polymerase Chain Reaction; SD, Standard Deviation; SGR, Specific Growth Rate; SR, Survival Rate; Vit-C, Vitamin C; Vit-E, Vitamin E; VHSV, Viral Hemorrhagic Septicemia Virus; WBC, White Blood Cell; WG, Weight Gain; YHV, Yellow Head Virus.

Keywords: Aquaculture, Immunostimulants, Fish microbes, Nile tilapia, Anti-stress, Dried ginger, Teak leaf

I. INTRODUCTION

Aquaculture addresses quickly developing food producing sectors. Intensive cultivating under high populace thickness give an approach to irresistible illnesses which represent a consistent and significant expense danger to aquaculture. The most ideal approach to conquer the sickness issues in a framework is through powerful management practices. As appropriate management is n't constantly conveyed, microorganisms become set up in creatures and produce infectious diseases. In this manner, rather than anti-microbials and chemotherapeutic agents, expanding consideration is being paid to the utilization of immunostimulants for infectious diseases control measures in aquaculture. One of the significant worries of aquaculture industry is the irresistible infections brought about by bacteria, viruses and parasites. The uncontrolled use of antibiotics, disinfectants and chemotherapeutics have been found to cause many of the diseases in fishes [7]. These unwanted changes in aquaculture industry affect the economy and upset the

improvement of significant fish delivering nations [6]. This has coordinated the consideration of researchers towards medicinal herbs, a promising source to yield bioactive compounds. Due to the wealth of these wide assortments of secondary metabolites, plants offer an incredible breadth to create as an answer for treating fish pathogens [3]. Phytochemicals or plant-derived active mixtures have been accounted for to have immunostimulatory, antimicrobial, growth increment, and anti-stress potential in aquaculture (Fig. 1) [11].

Utilization of costly chemo-therapeutants and anti-microbials bring:

• Gathering in the tissue as buildups or residues,
• Advancement of the resistance to drugs,
• Immuno-concealment and
• Decreased customer inclination for food fish treated with anti-biotics [1].

Immunostimulants and immuno-modulators involve a gathering of organic and synthetic modulators that upgrade the cellular and humoral protection mechanism in animals by upgrading its guard mechanism, Widely utilized for impeded resistant capacity, To settle the improved safe status (Fig. 2). The utilization of immunostimulants for the avoidance of sickness in fishes is considered as an appealing and promising region in the field of aquaculture [25].

A portion of its capacities incorporates

A. facilitate the capacity of phagocytic cells
B. increase their bactericidal properties
C. stimulate the common natural killer cells
D. stimulate supplement framework,
E. stimulate lysozyme action
F. Stimulate antibody responses

FAO (UN) has characterized that 'The advancement of moderate yet productive immunizations, the utilization of immunostimulants and non-specific immune enhancers, and the utilization of probiotics and bio-augumentation strategies for the improvement of aqatic environmental quality as significant zones for additional exploration in infectious prevention in aquaculture [16].

There are chiefly two unique kinds of immunostimulants viz. natural and synthetic one. Common immunostimulants comprises of Microbial subsidiary: β glucan, LPS, bacterins, Regular elements: Vit-C and Vit – E, Animal and Plant extracts, Polysaccharides (Chitin, Chitosan, Lentinan), Chemicals: (GH, Thyroid chemical, prolactin), Cytokines and others (ds RNA, recombinant proteins). Manufactured immunostimulant are Macrogard, Immersion grade, Aquasalor, Ergason (wealthy in polysaccharides), Lomal. Out of which most normally utilized immunostimulants in aquaculture are

Glucans. It is perhaps the most well-known immunostimulants which is gotten from the yeast cell wall and from certain higher plants. It has magnificent immuno-stimulatory properties and function admirably when infused or taken care of to the fish. Most normal brands sold are MacroGard, Vetregard and EcoActiva. Glucan ß-1,6, extended ß-1,3 Glucans were successful in animating the non-specific immune reaction in carp [26]. Incorporation of glucan in carp improved endurance, probably by means of incitement of both non-specific and specific immune responses (superoxide anion, IL-1 emission and counter acting agent development), paying little heed to how it was administered (intraperitoneal infusion, washing and oral organization) [23]. An incitement of complement and C reactive protein reactions were found in carp [18]. Investigations of glucan-initiated macrophages in trout uncovered an expanded capacity to slaughter salmonid microbe *Aeromonas salmonicida* [14].

Levamisole. It was a synthetic Imidazothiazole, broadly utilized in the human and veterinary medication as an enemy of helminthic agents. Levamisole is a viable treatment for Camallanus roundworm invasions in freshwater exotic fish. In fish, levamisole has been utilized in a couple of studies determined to improve the non-specific immune resistance reaction [17] or as an adjuvant with an antibody/ vaccine [13].

Chitin. Linear Beta-1,4-connected polymer of N-acetyl-D-glucosamine, A typical constituent of crustacean exoskeleton and parasitic cell walls. Commercially made from the shrimp and crab shells. As chitin is a non-harmful biodegradable and biocompatible substance, its subsidiaries are utilized in clinical practice [24]. Expanded resistance against *Aeromonas salmonicida* has been seen in brook trout when infused with chitin. Infusion of abalone concentrate and chitin, increment phagocytic reaction and natural killer cell activity in fish [20].

Chitosan. Chitosan is a linearhomopolymer of ß-(1,4)-2-amino-deoxy-D-glucose and is set up by the alkaline deacetylation of chitin get from shrimp and crab shell. Chitosan is utilized as an immunostimulant in aquaculture to ensure salmonids and carps against bacterial illnesses [2].

Natural plant products have been reported as anti-stress, growth improvement, appetite enhancer, tonic and immunostimulation, and to have aphrodisiac and antimicrobial properties in finfish and shrimp larviculture because of the presence of dynamic guideline parts like alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids, and fundamental essential oils [4, 15].

Fig. 1. Immuno-resistance properties of some Plants.

II. MATERIALS AND METHODS

A. Planning of diets

The segments of the fundamental control diet comprise of fishmeal (26%), com flour (21%), soybean supper (24%), wheat flour (6%), rice grain (16%), fish oil (0.5%), cellulose (5%) and a premix (1.5%). Five trial diets will be set up with 2.5 g/Kg, 5 g/Kg, 7.5 g/Kg, 10 g/Kg and 12.5 g/Kg of dried ginger and teak leaf powder. The powdered and sieved mixtures will be blended completely and pelletized utilizing a manual pelletizer. The pellets gathered in aluminum plate will be dried in a hot air oven at 50°C overnight. Subsequent to drying, the diets must be kept at room temperature (27°C) in an air impenetrable container.

B. Assortment and support of fish and test plan

Nile tilapia will be bought from concerned fisheries divisions like Marine Product Export Development Authority (MPEDA), and will be moved to the lab in circulated air through plastic sacks. Upon appearance, 240 tilapia must be haphazardly appropriated in twelve 150 liter tanks (20 fish for every tank) for about fourteen days acclimation. During acclimation, fish will be taken care of the control diet. Half of the water will be changed day by day with checking water quality.

The test configuration is as per the following:

(I) Control group (C) - Non-infected fish fed control diet without *P. hornemannii* improvement

(ii) T1 - Control diet + 2.5 g/kg dried ginger and teak leaf (half each)

(iii) T2-Control diet + 5 g/kg dried ginger and teak leaf (half each)

(iv) T3 - Control diet + 7.5 g/kg dried ginger and teak leaf (half each)

(v) T4 - Control diet + 10 g/kg dried ginger and teak leaf (half each)

(vi) T5 - Control diet + 12.5 g/kg dried ginger and teak leaf (half each)

Taking care of period will be for 30 days.

C. Testing

Toward the finish of taking care of period, three fish for every tank will be haphazardly chosen, and anesthetized by clove oil for immune response examination. Blood tests will be gathered in a vacutainer covered with EDTA and put away at - 20°C as indicated by Doan and co-authors [8, 9] until additional examination. For serum separation, blood samples kept at room temperature (27 °C) for one hour will be centrifuged at 2500 rpm for 10 min at 4°C as per Delannoy [5]. Liver and spleen will be gathered in trizol for RNA separation as per Yilmaz [21].

D. Development execution

Fish in every replication will be weighed at the end of the feeding trial. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and endurance rate/ survival rate (SR) will be determined utilizing the formulae:

(i) WG = last weight (g) - starting weight (g);

(ii) SGR (%) = 100 * (In definite or final weight - In
(iii) FCR = Feed given * (dried weight)/weight gain (wet weight)
(iv) SR (%) = (last fish number/starting fish number) × 100.

E. Challenge study

Aeromonas hydrophila will be cultured in Tryptic Soy Broth and incubated overnight at 37°C. The stock culture will be centrifuged for 10 minutes at 4500 rpm. The supernatant must be disposed of and the pellet ought to be re-suspended in clean saline arrangement. Optical thickness or density (OD456) of the solution will be changed to 0.5, which corresponds to 10^7cells/ml. Following 30 days of feeding trial experimentation, five fish from each tank must be haphazardly chosen, and tested with 0.1ml of saline solution of *A. hydrophila.* Challenge study goes on for 15 days and every day, mortality will denoted.

F. Hematological and immunological parameters

Hematological parameters like counts of Red Blood Cell (RBC) and White Blood Cell (WBC), Blood Hemoglobin (Hb) content, Packed Cell Volume (PCV) rate and differential leukocytes can be resolved utilizing a hemocytometer. The blood indices, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) can be resolved as portrayed by [8, 9, 22].

G. Immunological assays

Respiratory burst activity utilizing the protocol of Secombes [10] and assurance of myeloperoxidase action and serum lysozyme activities by the technique of Repel [12].

H. Gene expression

RT-qPCR investigation of gene expression will be led by the technique portrayed by Yılmaz and Ergün.

I. Statistical analysis

The information of every parameter was communicated as the mean standard deviation (SD) and the impacts of experimental diets were tested utilizing one-way analysis of variance (ANOVA). Contrasts were genuinely huge at $P < 0.05$.

beginning weight)/durationof investigation

III. RESULTS AND DISCUSSION

This venture targets exploring the capability of dried ginger and powdered teak leaf on Growth Improvement, immune-resistance, disease resistance, expression of antioxidant and immune cells and different hematological parameters in Nile tilapia. Assessment of the previously mentioned parameters help to break down the general wellbeing of fish and can propose the capability of this feed in fighting infectious diseases. This can be considered as a solid option for improving fish wellbeing by utilizing effectively accessible dried ginger and teak leaf. Here in this experimentation the highest cumulative survival was accomplished in fish fed with the addition of 2.5 g *Z. officinale* and *Tectona grandis* extract per kg of feed, trailed by fish in the treatment of 1.25 g/kg of feed. At higher doses, survival seemed to diminish, and surprisingly had the same value as that of control fish. This outcome demonstrated that if the fish are fed with the addition of *Z. officinale* and *Tectona grandis* extract with a concentration that is too high, at that point endurance or survival decrease. For this situation, the excessive doses will have an immunosuppression effect that suppresses the immune system of the fish (Sakai 1999). Resistance of fish was expressed as survival rate accomplished after challenge test with *A. hydrophila* suspension having 5×10^7 cfu/mL. Mortality was seen as long as 8 days post-challenge. In all treatment, mortality started to occur on day two post challenged and proceeded until day seven. The result of variance analysis showed that the addition of *Z. officinale* and *Tectona grandis* extract in feed had good impact on the resistance of *O. niloticus* against *A. hydrophila* ($p<0.05$). The highest survival rate was obtained in fish fed pellet with addition of *Z. officinale* and *Tectona grandis* extract of 2.5 g/kg of feed which reached 77.4% while in the untreated fish the survival rate was found to be 44.3%. In light of Duncan's Test, survival rate on treatment of 2.5 g/kg treated feed was significantly different from control fish ($p<0.05$). There was no huge contrast observed between fish fed pellet supplemented with *Z. officinale* and *Tectona grandis* extract at 1.25, 5, 10 g/kg feed and control (Table 1).

Table 1: Resistance of *Oreochromis niloticus* after challenged with *Aeromonas hydrophila.*

Ginger and teak leaf extracts (g/kg of pellet)	**Resistance in %**
0	44.3 ± 12.5
1.25	56.7± 6.5
2.5	77.4 ± 6.5
5	43.3 ± 6.5
10	54.3 ± 6.5

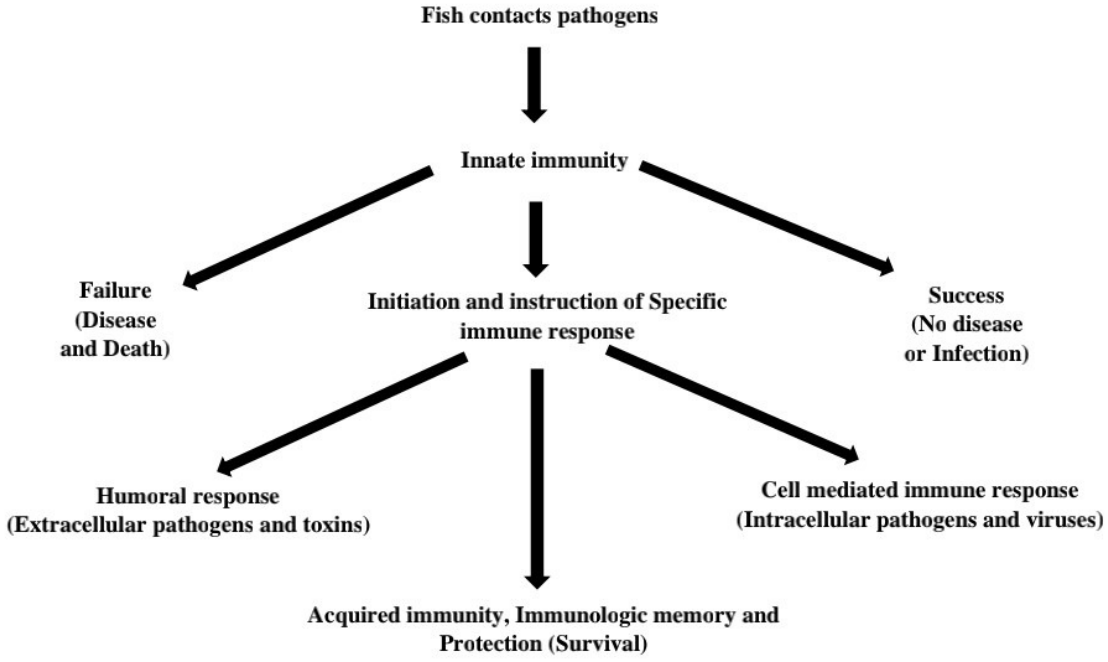

Fig. 2. Immune response of fishes following encounter with a pathogen.

Table 2: Changes in serum electrolytes and immunoglobulin gM of *O. niliticus* after using different doses of ginger and teak leaf extracts (2.5, 5, 7.5, 10, 12.5 g/Kg).

Dose/parameters	Control	Ginger and Teak 2.5 g/kg	Ginger and teak 5 g/kg	Ginger and teak 7.5 g/kg	Ginger and teak 10 g/kg	Ginger and teak 12.5 g/kg
Na mmol/ L	151 ± 3	155 ± 4^{+}	158 ± 2^{+}	159 ± 4^{++}	156 ± 3^{+}	157 ± 5^{+}
K mmol/ L	5 ± 0.25	5.5 ± 0.3^{+}	5.7 ± 0.33^{++}	5.95 ± 0.4^{++}	5.5 ± 0.3^{+}	5.65 ± 0.4^{++}
Ca mmol/ L	12 ± 1	12.3 ± 2^{+}	12.6 ± 1^{+}	12.9 ± 2^{++}	12.6 ± 1^{+}	12.7 ± 2^{++}
IgM value (µg/ml)	27.5 ± 0.5	30.1 ± 0.1^{+}	32.2 ± 0.2^{++}	33.1 ± 0.3^{+++}	38.0 ± 0.7^{+++}	41.0 ± 0.6^{+++}
+ significant at p< 0.5, ++ highly significant at p< 0.01, +++ very highly significant at p< 0.001 (There was significant difference between treated group and control group)						

Table 3: Changes in serum protein, lipid content, glucose of *O. niloticus* after using different doses of ginger and teak leaf extracts (2.5, 5, 7.5, 10, 12.5 g/Kg).

Doses/parameters	Control	Ginger and Teak 2.5 g/kg	Ginger and teak 5 g/kg	Ginger and teak 7.5 g/kg	Ginger and teak 10 g/kg	Ginger and teak 12.5 g/kg
Total protein (g/dl)	6.2 ± 1.3	6.3 ± 0.2	6.8 ± 0.4^{+}	6.9 ± 0.7^{+}	6.9 ± 0.8^{++}	7.0 ± 0.9^{++}
Total lipid	4.7 ± 0.2	4.4 ± 0.3	4.3 ± 0.5^{+}	4.2 ± 0.1^{+}	4.0 ± 0.2^{++}	4.0 ± 0.2^{++}
Glucose (mg/dl)	99.8 ± 1.2	99.8 ± 1.6	98.1 ± 1.2^{+}	97.0 ± 1.4^{+}	96.1 ± 1.3^{++}	96.2 ± 1.1^{++}
Cholesterol (mg/dl)	189 ± 11	187 ± 13	183 ± 11^{++}	170 ± 15^{+++}	168 ± 22^{+++}	168 ± 21^{+++}
Triglycerides (mg/dl)	3.10 ± 0.3	3.9 ± 0.3	3.7 ± 0.3^{+}	3.71 ± 0.2^{+}	3.12 ± 0.3^{++}	3.0 ± 0.6^{++}
+ significant at p< 0.5, ++ highly significant at p< 0.01, +++ very highly significant at p< 0.001 (There was significant difference between treated group and control group)						

It was clear to note that the level of Ca, Na and K and immunoglobulin (IgM Value) in plasma were increased dramatically with the increasing levels of ginger and teak leaf (Table 2). The results showed increment in the level of total protein in the group fed on diet containing different doses of ginger and teak leaf extracts (2.5, 5, 7.5, 10, 12.5 g/Kg). The mean values of total plasma lipids showed a decline similar to cholesterol and

triglycerides in the fed group compared to the control ones (Table 3).

The results showed the significant difference in the count of red blood cells (RBCs), hemoglobin content, platelets and hematocrit after using different doses of ginger and teak leaf in the feed. It was clear that both hemoglobin content and Erythrocyte count increased on the diet increment with ginger and teak leaf (Table 4).

Table 4: Changes in the counts of red blood cells (RBCs), hematocrit HCT, platelets and hemoglobin (Hb) of *O. niloticus* after using different doses of ginger and teak leaf extracts (2.5, 5, 7.5, 10, 12.5 g/Kg).

Doses/parameters	Control	Ginger and Teak 2.5 g/kg	Ginger and teak 5 g/kg	Ginger and teak 7.5 g/kg	Ginger and teak 10 g/kg	Ginger and teak 12.5 g/kg
RBCs ($x10^6$ / µL)	1.4 ± 0.3	1.6 ± 0.4	$1.71 \pm 0.4^{+}$	$2.0 \pm 0.3^{++}$	$2.53 \pm 0.2^{++}$	$2.61 \pm 0.2^{+++}$
HCT (%)	23.2 ± 1.1	26.2 ± 1.4	$27.3 \pm 1.3^{+}$	$28.7 \pm 2.1^{+}$	$29.8 \pm 1.4^{++}$	$29.2 \pm 2.0^{++}$
Platelets 10^3 mm^{-3})	286 ± 1.2	286 ± 1.2	$291 \pm 1.1.2^{+}$	$293 \pm 1.2^{++}$	$296 \pm 1.2^{+++}$	$298 \pm 1.2^{+++}$
Hb (g/ dL)	6.71 ± 1.1	7.32 ± 1.4	$7.78 \pm 1.4^{+}$	$8.2 \pm 1.4^{+}$	$8.13 \pm 1.0^{+}$	$8.7 \pm 1.5^{++}$
+ significant at p< 0.5, ++ highly significant at p< 0.01, +++ very highly significant at p< 0.001 (There was significant difference between treated group and control group)						

IV. CONCLUSION

Here the results of investigation proved that dried ginger and teak leaf can enhance the non-specific immunity in Nile tilapia. Supplementation of *Z. officinale* and *Tectona grandis* into fish feed improved resistance of fish against *A. hydrophila* infection. Ginger has the great power of stimulating the immune system of the fish. The teak leaf helps to enhance coloration of fishes and provides positive effects on scale strengthening. It can also maintain the water pH level and act like a natural antibiotic. Some of the fungal diseases and bite injuries were well treated with this. By combining this two ingredients to the fish diet had very good results in the immuno- resistance and healthiness of *Orechromis niloticus.*

V. FUTURE SCOPE

The role of immunostimulants in aquaculture has very much importance as the aquatic life is concerned. It had reasonable role in boosting the immune system of the fish and thus farmer can ensure a better production. In future definitely researchers can find more and more immunostimulants which may be derived either from natural sources or synthetic, which can improve the health of fish. Here the results of investigation proved that dried ginger and teak leaf can enhance the non-specific immunity in Nile tilapia by improving the growth.

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