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Effect of Nucleotide on Growth, Immune Responses and Resistance of *Labeo Rohita* to *Aeromonas Hydrophila* Infection

Abstract

A 60-day feeding trial was conducted to evaluate the effect of dietary nucleotide on growth, survival, immunity and resistance to *Aeromonas hydrophila* infection in rohu (*Labeo rohita*). The nucleotide was supplemented at 0, 5, 10 and 15 g/kg diet. The test diets were fed for 60 days in triplicate groups of fish, which had initial weight of 1.3 g. At the end of the feeding trial, growth was recorded and non-specific immune parameters, such as; superoxide anion production and total serum protein were studied in blood samples. Total serum protein and superoxide anion production were significantly ($P<0.05$) higher in fish fed nucleotide-based diets. The relative percent survival of fish after the challenge test against *Aeromonas hydrophila* disease was significantly ($P<0.05$) higher in fish fed nucleotide-incorporated diets.

Keywords: Nucleotide; Immune response; *Labeo rohita; Aeromonas hydrophila*; Disease resistance

Research Article

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Abbreviations: CRD: Completely Random Design; NBT: Nitro Blue Tetrazolium; LD_{50}: Lethal Dose 50%; RPS: Relative Percent Survival; ANOVA: Analysis of Variance; BKC: Benzalkonium Chloride

Introduction

Immunostimulants have promising beneficial effects in aquaculture. They are known to enhance growth and disease resistance in fish [1]. Dietary supplements, including killed bacteria, *β*-glucans, lipopolysaccharides, and nucleotides have exhibited immunostimulatory effects [2]. Dietary nucleotides have been shown to have several beneficial effects in humans [3,4] as well as aquatic animals [5,6]. Other benefits derived from administration of nucleotides include rapid intestinal repair [7], improved mucosal gut flora and mucosal surfaces [8,9] and elongation of the intestinal tract [10,11] in aquatic animals. Nucleotides also have been shown to enhance the immune system [12] and disease resistance of various animals [13,14]. Most cell types can synthesize nucleotides from purines and pyramidines. However, denovo synthesis and salvage pathways are metabolically expensive processes. Further, sufficient quantities of nucleotides required to meet the metabolic requirements are presumably not synthesized by fish under stressful conditions [5]. Additional sources of exogenous nucleotides in the diet will help to optimize function of rapidly dividing tissues [10] and may enhance feed intake [11], increase growth rate [15], and elevate immune responses [16]. The objective of the present investigation was to study the effect of nucleotide on growth, survival, immune responses and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*.

Materials and Methods

Labeo rohita fingerlings with an average weight of 1.3g were acclimatized by feeding control diet for 2 weeks. Rohu were randomly distributed into 4 groups: control (T_0) (basal diet); T_1 (basal diet +5g/kg Nucleotide); T_2 (basal diet + 10g/kg Nucleotide); and T_3 (basal diet + 15g/kg Nucleotide) were arranged in triplicate groups following a completely random design (CRD) design. The total volume of the water in culture tanks was maintained at 100 L level throughout the experimental period. Round the clock aeration was provided. Feed was given at 4% body weight for 60 days twice a daily at 10:00 and 17:00 hr. Uneaten feed and faecal matter were siphoned out daily and 80% water was replaced with freshwater.

Experimental diet

The composition of the formulated experimental diet is given in Table 1. Vitamin-mineral pre- mix was added after cooling and the dough was extruded through a pelletizer having 2 mm dia. The pellets were dried in hot air oven at 60°C till the moisture content was reduced to less than 10%. Diets were packed separately in high density polythene bags.

Growth trial

Labeo rohita fingerlings were weighed at the beginning and every 15 days interval thereafter till the completion of the experiment. The growth performance of rohu was evaluated in terms of weight gain. Survival was calculated as difference between the numbers of live animals stocked at the beginning and survived at the end of the experiment (Table 2).

Table 1: Formulation of nucleotide supplemented diets (%).

Ingredient	Treatment			
	T_0	T_1	T_2	T_3
Soya Bean Meal	47.95	47.95	47.95	47.95
Ground nut oil cake	20	20	20	20
Rice Bran	26.05	26	25.05	24.55
Tapioca Flour	5	5	5	5
Vitamin and Mineral Mixture	1	1	1	1
Nucleotide	0	0.5	1	1.5
Total	100	100	100	100

Table 2: Growth performance and survival (mean ± SD) of rohu fed different levels of nucleotide for 60 days.

Items	T_0	T_1	T_2	T_3
Final Weight Gain (g fish-1)	3.55±0.11[a]	3.57±0.16[a]	3.88±0.14[b]	3.57±0.12[a]
Weight Gain (% of initial weight)	273.58±8.58[a]	275.07±12.02[a]	298.46±11.61[b]	274.61±8.87[a]
Survival Rate (%)	62.66±4.61[a]	64.0±6.93[a]	72.0±8.0[a]	65.33±6.11[a]

Different superscripts in the same row indicate significant difference (P<0.05) among control and treatment groups; Ducan's multiple range test P= 0.05; The value expressed as a mean ± SD.

Immune responses

Nitro blue tetrazolium (NBT) assay: The activated phagocytes (neutrophil and macrophages) are characterized by their ability to adhere to glass or plastic and produce oxygen free radicals. NBT in its reaction with oxygen free radicals is reduced to blue formazan, the extent of which can be determined spectrophotometrically. The results were read on an ELISA reader at 620 nm using KOH and DMSO mixture as blank.

Total serum protein: Total serum protein was measured by using GeNei™ protein analysis kit (by Lowry's method) (Table 3).

Table 3: Effect of nucleotide on Nitro blue tetrazolium assay and total serum protein in different treatments and control group after feeding trial.

Items	T0	T1	T2	T3
Superoxide anion production (OD at 630 nm)	0.05±0.002[a]	0.07±0.004[ab]	0.084±0.011[b]	0.074±0.007[ab]
Total serum protein (mg/ml)	42.83±1.04[a]	46.5±1.32[b]	49.0±0.5[b]	40.73±3.356[a]

Different superscripts in the same row indicate significant difference (P<0.05) among control and treatment groups; Ducan's multiple range test P= 0.05; The value expressed as a mean ± SD.

Challenge study

LD50: *A. hydrophila* isolate was tested for pathogenecity in fingerlings of rohu maintained in aquarium tanks (20L) with aeration. Fish were injected with 0.1ml. *A. hydrophila* inoculate ranging from 10^2 to 10^{10} CFU/ml. Ten fish were used for each treatment. Mock injection was given to control groups with sterile PBS. Mortalities were recorded daily for 10 days and the lethal dose 50% (LD_{50}) calculated.

The susceptibility of *Labeo rohita* to *Aeromonas hydrophila* infection: Fish were injected intramuscularly with a 24 hour old culture of *A. hydrophila* (10^6 CFU/ fish, LD_{50} dose). The susceptibility was conducted for 5 days. A minimum of 21 fish per treatment in triplicate groups were challenged at 60 days post treatment. Challenged fish were maintained in FRP tanks. Appearance of gross clinical lesions and mortality pattern if any, were observed during the study. The cause of mortality was further confirmed by reisolating the organism from moribund or dead fish kidney or Rimler Shot's medium (Himedia, India). Relative percent survival (RPS) was calculated according to Amend [17].

RPS =[1-(% mortality of treatment group / % mortality in control)] x 100

The mean values of all the parameters were analyzed by one-way analysis of variance (ANOVA). Comparisons were made at 5% probability level by using statistical package SPSS, Version 20. Duncan's multiple range tests was used to determine the significant difference between the control and treatment means (Table 4).

Table 4: Relative percentage of survival (RPS) of *Labeo rohita* recorded in different treatments and control group after challenged with *Aeromonas hydrophila.*

Treatment	Cumulative Percentage of Survivality	Relative Percentage of Survival (RPS) Against Control
T_0	14.28^a	-
T_1	33.33^b	22.22^a
T_2	52.38^c	44.44^b
T_3	38.09^b	27.78^a

Different superscripts in the same row indicate significant difference (P<0.05) among control and treatment groups; Ducan's multiple range test P<0.05; the value expressed as a mean ± SD.

Results and Discussion

Effect of nucleotide on growth and survival of *Labeo rohita*

The final weight of rohu fed with supplemental nucleotides was found to be greater than that of rohu fed the basal diet. Significant (P=0.052) difference in growth was observed between rohu fed with nucleotide 10 g kg^{-1} diet (Table 2) and control. Rohu had attained mean weight of 298.46% in T_2 group, which is 24.88% higher growth than the groups fed control diet. Highest survival was recorded in T_2 (72.0%). No significant difference (P=0.125) was observed in survival rate due to the inclusion of nucleotide in fish diets. The survival of rohu ranged from 62.66 to 72.0% overall. Dietary supplementation of nucleotide showed positive effect on weight gain and survival. Our results were supported by the findings of Burrells C [6], who reported that supplementation of fish feeds with nucleotides shows positive effect on growth.

Effect of nucleotide on immune responses

Superoxide anion production (NBT) and total serum protein of fish fed nucleotide incorporated was significantly higher (P<0.05) than that of control diet. The results are supported by the findings of earlier workers [10,18,19]. Levamisole used as an immunostimulant promoted recovery from immunosuppression [2,20] and also enhanced both innate and specific humoral and cellular immune responses [21,22]. Benzalkonium chloride (BKC) was used as an effective antibacterial and immunostimulant for *M. rosenbergii* [23]. Lee & Shiau [24] reported that intracellular superoxide anion production of the haemocytes in *P. monodon* fed diets containing ascorbate supplements was approximately higher than that of shrimp fed un-supplemented diets. The haemoglobin content in the blood and oxygen consumption increases when fishes are under stress. Under such conditions, there will be an increase in release of immature RBCs from the haemopoietic organs, which in turn elevate haemoglobin concentration in blood [25].

Effect on infection of *Aeromonas hydrophila* infection

In our investigation, control group showed higher mortality compared to treatment groups. The relative per cent survival of rohu after the challenge study was significantly higher in rohu fed with nucleotide-incorporated diets, which is supported by the earlier findings [10]. Supplementation of dietary ribonucleic acid at 0.4% enhances the relative per cent survival in *Labeo rohita* against *Aeromonas hydrophila* infection [26].

Conclusion

It can be concluded that present investigation indicated that the inclusion of nucleotide at 10g kg^{-1} in diet improve growth performance and enhance the immune response as well as disease resistance in rohu.

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