E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(5): 243-246

www.fisheriesjournal.com
Received: 01-07-2017
Accepted: 02-08-2017

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Effect of organic selenium on growth, immune response and resistance of *Labeo rohita* to *Aeromonas hydrophila* infection

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Abstract
A 60 day feeding trial was conducted to evaluate organic selenium supplementation in the diet of fingerling Indian major carp, *Labeo rohita*. A basal diet was formulated to contain 30% protein. Three levels of organic selenium (1, 2 and 3 g kg^{-1} diet) were added to the basal diet. Each diet was fed to the 3 replicate groups of fingerling *Labeo rohita* twice a daily @ 5% of the body weight. After the feeding trial of 60 days an *Aeromonas hydrophila* challenge study was conducted to test the effects of diet on disease resistance. NBT test and total serum protein were carried out to assess the immune response. The weight gain%, survival, NBT levels and Relative Percent survival (RPS) were significantly higher in *Labeo rohita* fingerlings fed with organic selenium incorporated diets than in control groups.

Keywords: organic selenium, immune response, *Labeo rohita*, *Aeromonas hydrophila*

1. Introduction
In recent years, increased importance has been given to health management in aquaculture. Use of drugs and antibiotics is discouraged in aquaculture in view of residual accumulation, destruction of gut micro flora, development of resistant bacteria and other various harmful effects. Indiscriminate use of drugs and chemicals, mostly based on presumptive diagnosis can lead to problems like degradation of aquatic ecosystems (Rao *et al*. 1992) [16], accumulation as tissue residue in cultured fish (Ellis *et al.* 1988) [5], immunosuppression in cultured fish species (Muiswinkel *et al*. 1985) [12] and the development of drug resistance pathogens. Hence, disease management in aquaculture need to focus on preventive measures related to water quality and husbandry practices. The level of resistance to infection in the cultured organisms should be increased to reduce the risk of disease. This can be achieved through the use of efficient feeds, vaccines, immunostimulants and immunemodulators to enhance disease resistance (Raa *et al.* 1992, Blaut 2002) [15, 4].
Selenium (Se) is one of the trace mineral which has recently received a considerable amount of attention in animal nutrition. Selenium is a component of the enzyme glutathione peroxidase (GPx, EC 1.11.1.9) (Rotruck *et al*., 1973) [17]. This enzyme catalyzes reactions necessary for the conversion of hydrogen peroxide and fatty acid into water and fatty acid alcohol by using reduced glutathione, thereby protecting cell membranes against oxidative damage. Organic Se (selenomethionine) has been reported to have higher bioavailability than the inorganic Se (sodium selenite) for Atlantic salmon (Bell and Cowey, 1989; Lorentzen *et al*., 1994) [2, 11] and channel catfish (Wang and Lovell, 1997) [14].
The objective of the present investigation was to study the effect of organic selenium on growth, immune responses and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*

2. Materials and Methods
Labeo rohita fingerlings with an average weight of 1.2g were used. The fingerlings were acclimatized by feeding control diet for 2 weeks. *Labeo rohita* fingerlings (300) were randomly distributed into 4 groups (1 control and 3 treatment groups): control (T0) (basal diet); T_1 (basal diet +1.0 g/kg OS); T_2 (basal diet + 2.0 g/kg OS); and T_3 (basal diet + 3.0 g/kg OS) were arranged in triplicates 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 fecal matter were siphoned out daily and 80% water was replaced with freshwater.

2.1 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.

Table 1: Formulation of organic selenium based test diets

Treatment				
Ingredient	**T_0**	**T_1**	**T_2**	**T_3**
Soyabean meal	47.95	47.95	47.95	47.95
Ground nut oil cake	20.0	20.0	20.0	20.0
Rice bran	26.05	25.95	25.85	25.75
Tapioca flour	5.0	5.0	5.0	5.0
Vitamin & mineral mixture	1.0	1.0	1.0	1.0
Organic selenium	0.0	0.1	0.2	0.3
Total	100	100	100	100

2.2 Growth Trial

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

2.3 Effect of Organic Selenium on Immune Responses

2.3.1 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.

2.3.2 Total serum protein

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

2.4 Effect on infection with *Aeromonas hydrophila*

2.4.1 LD_{50}

A. hydrophila isolate was tested for pathogenicity in fingerlings of *L. rohita* maintained in aquarium tanks (20L) with aeration. Fish were injected with 0.1ml each, *A. hydrophila* inoculate ranging from 10^2 to 10^{10} CFU/ml. Ten fish were used for each dose. 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.

2.5 The susceptibility of *Labeo rohita* to *Aeromonas hydrophila* infection

Labeo rohita were challenged 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 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 (1981) [1].

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 made at 5% probability level by using statistical package SPSS, Version 16. Duncan's multiple range tests was used to determine the significant difference between the control and treatment means.

3. Results

3.1 Effect of organic selenium on growth and survival of *Labeo rohita*

Water quality parameters recorded during the experimental period were in the acceptable range. The weight gain% and survival was found significant (P<0.05). The highest weight gain was recorded in T_1 (3.51g). Rohu had attained a mean weight of 292.76% in T_1 group, which is 7.49 % higher growth than the groups fed control diet. (Fig 1)

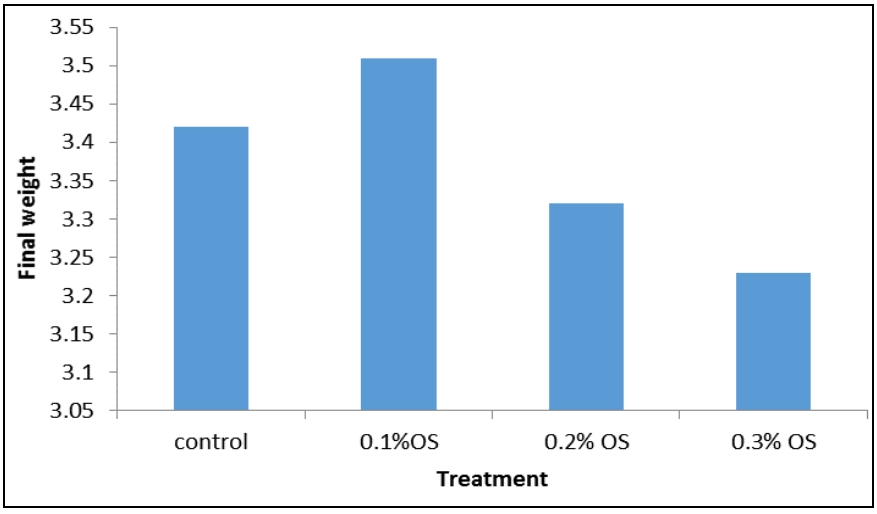

Fig 1: Final weight gain of *Labeo rohita* fed diets containing graded levels of organic selenium for 60 days in a feeding trial

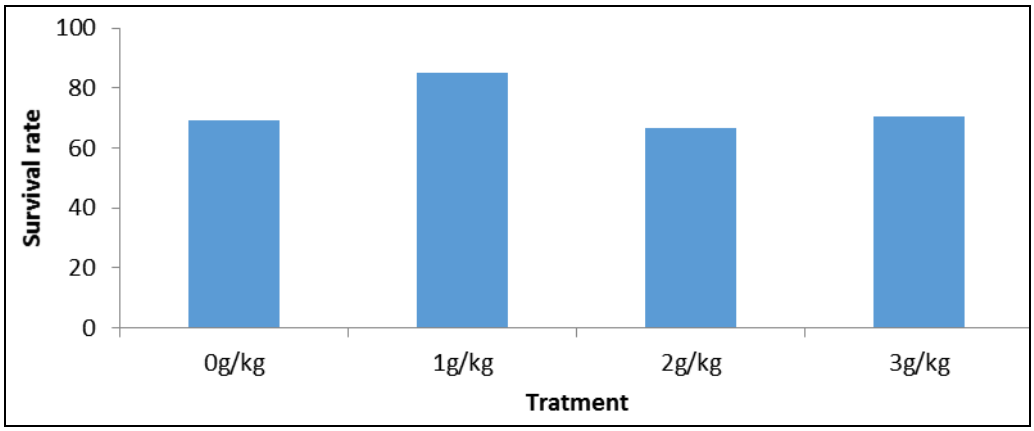

Fig 2: Survival rate% of *Labeo rohita* fed diets containing graded levels of organic selenium for 60 days in a feeding trial

Highest survival was recorded in T_1 (1g kg^{-1}) (P=0.125) (Fig 2). No significant difference was observed in survival rate due to the inclusion of OS in fish diets. The survival of rohu ranged from 69.0 to 85.0%. Supplementation of organic selenium enhanced the survival of rohu.

3.2 Effect of organic selenium on immune responses

Table 2: Effect of organic selenium on Nitro blue tetrazolium assay and total serum protein in different treatments and control group after feeding trial

Items	Organic selenium levels (g/kg diet)			
	T_0	T_1	T_2	T_3
Superoxide anion production (OD at 630 nm)	0.067 ± 0.007^{a}	0.084 ± 0.005^{b}	0.071 ± 0.003^{a}	0.067 ± 0.004^{a}
Total serum protein (mg/ml)	43.56 ± 2.14^{ab}	48.5 ± 1.32^{b}	37.5 ± 1.32^{a}	38.33 ± 1.89^{a}

Different superscripts (ab) in the same row indicate significant difference (P<0.05) among control and treatment groups (T_0, T_1, T_2 and T_3); Ducan's multiple range test a = 0.05; The value expressed as a mean ± SD.

3.3 Effect on infection of *Aeromonas hydrophila* infection

The disease challenge against *Aeromonas hydrophila* infection resulted in 85.72% mortality of fish fed the control diet. The relative percent survival of fish fed diet containing organic selenium in T_1 (44.4%) was significantly (P<0.05) higher (Table 3).

Table 3: Relative percentage of survival (RPS) of *Labeo rohita* recorded in different treatments and control group after challenged with *Aeromonas hydrophila*

Treatment	Realative percentage of survival (RPS) against Control
T_1	44^{c}
T_2	33^{b}
T_3	23^{a}

Different superscripts (abc) in the same row indicate significant difference (P<0.05) among control and treatment groups (T_0, T_1, T_2 and T_3); Ducan's multiple range test a, 0.05; the value expressed as a mean ± SD

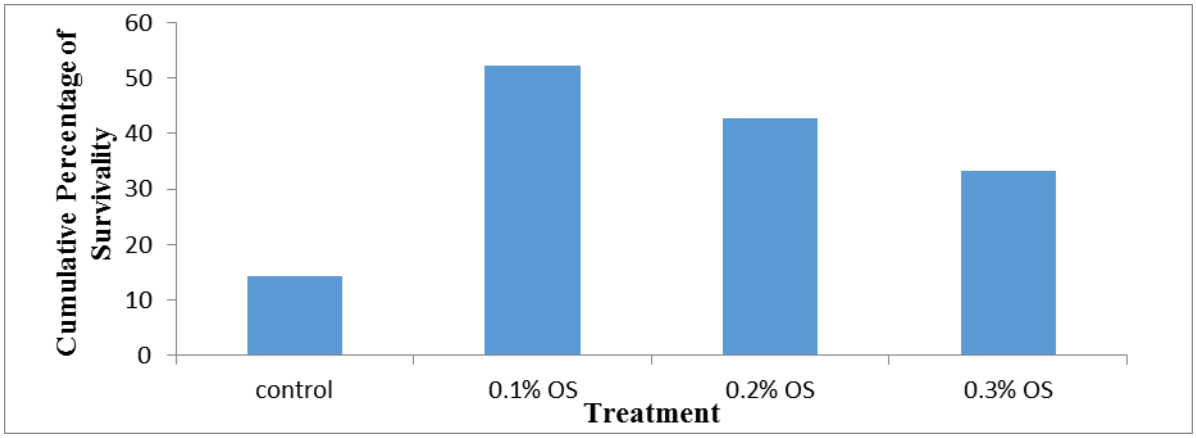

Fig 3: Cumulative percentage of survivality of *Labeo rohita* recorded in different treatments and control group after challenge against *Aeromonas hydrophila*

Discussion

Dietary supplementation of organic selenium showed effect on weight gain and survival. Present results were in accordance with the findings of Lin and Shiau (2005) [10] reported that Grouper have a requirement for Se that cannot be met by Se in the unsupplemented diet, thus dietary supplementation is necessary. Selenium deficiency generally results in growth depression. Mortality noted in salmon fry fed a selenium-deficient diet was prevented by administration of a diet containing 0.1 mg Se kg^{-1} and 500 IU vitamin E kg^{-1}

(Poston *et al*., 1976) [14].

Nitro blue tetrazolium assay and total serum protein of fish fed organic selenium incorporated was significantly higher (P<0.05) than that of control diet. This is supported by the findings of Lin and Shiau (2007) [6] they found that high dietary Se (1.6 mg kg^{-1} diet) supplementation reduced the oxidative stress and improved the fish immune response *Epinephelus malabaricus.*

Selenium deficiency causes growth depression in rainbow trout (Hilton., 1980) [8], carp (Satoh *et al*., 1983) [19] and catfish (Gatlin and Wilson, 1984) [6] but the selenium deprivation alone does not produce any pathological sign in these fish. Glutathione peroxide activity in plasma and liver decrease during selenium deficiency (Poston *et al*., 1976; Hilton *et al*., 1980; Bell *et al*., 1985; Gatlin *et al*., 1986) [14, 8, 3, 7].

In our investigation, control group showed higher mortality compared to treatment group. Relative percent survival was significantly higher in the treatment groups fed with 0.1% levels of organic selenium. In recent years, several reports indicated that oral administration of nucleotides enhanced immune responses and/or disease resistance in several fish species. Supplementing 0.2 g kg^{-1} of Sel-Plex, which equates to approximately 0.4 mg kg^{-1} OS in the diet of Marron, is recommended to enhance growth performance, survival and disease resistance against *V. mimicus* (Rudy and Fotedar, 2013) [18].

Conclusion

The overall outcome of the present investigation suggested that the inclusion of organic selenium @ 1g kg^{-1} in diet, will improve growth performance and enhance the immune response as well as the relative percent survival of *Labeo rohita* fingerlings. These results may be useful for the farmers to improve the fish production per unit area.

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