Indian Journal of Animal Sciences **82** (7): 779–782, July 2012

Effect of brewer's yeast (*Saccharomyces cerevisiae*) on growth, survival and immune response in *Labeo rohita*

AMIR MOHSENI[1], H SHIVANANDA MURTHY[2], E G JAYARAJ[3], R SHANKAR[4] and C S TEJPAL[5]

Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore, Karnatka 575 002 India

Received: 26 May 2011; Accepted: 26 February 2012

ABSTRACT

A 120-day feeding trial was conducted to evaluate brewer's yeast supplementation in the diet of juvenile Indian major carp, *Labeo rohita*. A basal diet was formulated to contain 30% protein and 7–8% fat. Three levels of brewer's yeast (1, 2 and 3 g kg^{-1} diet) were added to the basal diet. Each diet was fed to the 3 replicate groups of juvenile *Labeo rohita* twice a daily @ 5% of the body weight. After the feeding trial of 120 days an *Aeromonas hydrophila* challenge study was conducted to test the effects of diet on disease resistance. Lysozyme activity and NBT test were carried out to assess the immune response. The weight gain %, survival %, Lysozyme activity, NBT levels and RPS were significantly higher in *Labeo rohita* fingerlings fed with brewer's yeast incorporated diets than in control groups.

Key words: *Aeromonas hydrophila,* Brewer's yeast, Immune response, *Labeo rohita*, *Saccharomyces cerevisiae*

In 2006, the total production of freshwater fishes was 2.47 million tonnes, of which 87% was contributed by carps (FAO 2006). Polyculture with catla, rohu and mrigal and monoculture, particularly with rohu, is also practiced. Rohu enjoys high consumer preferences in many states Murthy (2002). Indiscriminate use of drugs and chemicals, mostly based on presumptive diagnosis can lead to problems like degradation of aquatic ecosystems (Rao *et al*. 1992), accumulation as tissue reside in cultured fish (Ellis *et al.* 1988), immunosuppression in cultured fish species (Muiswinkel *et al*. 1985) and the development of drug resistance in pathogens of human health significance. 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 use of efficient feeds, vaccines, immunostimulants and immunomodulators to enhance disease resistance (Raa *et al.* 1992, Blaut 2002). Vaccines were developed against several bacterial diseases in fish (Lillehaug 1989).

Yeast by-products from the brewing industry are natural diet additives that have positively influence on non-specific immune responses (Siwicki *et al*. 1994, Anderson *et al*. 1995) as well as growth (Rumsey *et al*. 1991, Oliva-Teles and Goncalves 2001) of some fish species. However, effect of yeast products has not been investigated in rohu (*Labeo rohita*). In addition, doses and time of administration have been recognized to have important effects on immuno-stimulant function. Further efficacy of oral administration of immunostimulants reportedly decreased over time (Sakai *et al*. 2001).

Present address: [1,4,5]Students (e mail[1]: mohseni_fishery @yahoo.com), [2](Professor and Head (hsmurthy05@yahoo.com), [3]Professor (jaya_ettigi@yahoo.com) Department of Aquaculture, College of Fisheries.

The objective of the present investigation was to study the effect of brewer's yeast on growth, survival, immune responses such as lysozyme, bacteriocidal hemolytic activities and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*.

MATERIALS AND METHODS

Labeo rohita fingerlings with an average weight of 5±0.05 g 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 (T_0) (basal diet); T_1 (basal diet +1.0 BY); T_2 (basal diet + 2.0 BY); and T_3 (basal diet + 3.0 BY) 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 5% body weight for 120 days twice a daily at 10:00 and 18:00 hr. Uneaten feed and faecal matter were siphoned out daily and 80% water was replaced with freshwater.

Experimental diet: The composition of the formulated

Table 1. Formulation of nucleotide based test diets

Ingredient (g/kg)	Treatment			
	T_0	T_1	T_2	T_3
Fish meal	250	250	250	250
Groundnut oilcake	400	399	398	397
Rice bran	200	200	200	200
Tapioca flour	70	70	70	70
Sardine oil+ soybeen oil	60	60	60	60
Vit-Min mixture	20	20	20	20
Brewer's yeast	0.0	1. 0	2.0	3.0

experimental diet is given in Table 1. Vitamin-mineral premix was added after cooling and the dough was extruded through a pelletizer having 2 mm diameter. 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 start and every 15 days interval thereafter till the termination of the experiment on the 120th day. The growth performance of *Labeo rohita* fingerlings was evaluated in terms of weight gain. Survival of *Labeo rohita* fingerlings calculated as difference between the number of live animals stocked at the beginning and survived at the end of the experiment.

Effect of brewer's yeast on 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.

Lysozyme activity: Twelve hour old culture of *Micrococcus* sp. was suspended in 0.05 M sodium phosphate buffer, to give a transmittance of 40% at 450 nm. The blood was collected from fish and centrifuged at 9000 rpm for 3 min to settle RBCs and white blood cells (WBC). The supernatant containing lysozyme was used for the assay. To 2.2 ml of *Micrococcus* suspension in a cuvette 0.3 ml of supernatant was added. The increase in transmittance at 450 nm was measured every 15 min for 1 h spectrophotometrically. A 25% change in transmittance indicated that substantial amount of bacteria had been lysed.

Effect on infection with Aeromonas hydrophila

LD_{50}: The control fishes were transferred to aquaria and fed with control diet to determine LD_{50} at 120 days. Five individuals each from control and treatments were challenged with graded densities (10^4, 10^5, 10^6, 10^7 and 10^8 cfu/ml @ 0.1 ml/fish) of virulent *A. hydrophila* (24 h TSB culture).

Challenge study with Aeromonas hydrophila*:* Experimented fish were injected intra muscularly with a 24 h culture of *A. hydrophila* (10^6 cfu/fish). A minimum of 21 fish per treatment in triplicate were challenged for 120 days post treatment. Challenged fish were maintained in well aerated aquarium tanks. Dead fish were tested for *A. hydrophila* positivity by re-isolating the pathogen from the kidney on Rimler Shot's medium. Post challenge mortalities were recorded for 7 days. Only specific mortalities were used in evaluating the potency of injection (10^6 cfu/fish) challenges. Relative percent survival (RPS) was calculated according to Amend (1981).

Confirmation of Aeromonas hydrophila *infection:* Specific mortalities were ascertained by re-isolating the pathogen from the kidneys of the diseased fish.

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 12. Duncan's multiple range tests was used to determine the significant difference between the control and treatment means.

RESULTS AND DISCUSSION

Effect of brewer's yeast 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 ($P<0.05$). A clear trend of increase in weight gain was observed due to gradual supplementation of brewer's yeast. The highest weight gain was recorded in T_3 (76.20 g) (Figs 1, 2).

Supplementation of brewer's yeast enhanced the survival of rohu. Higher survival rates were recorded in all the treated groups than in control (Fig. 2). In the present study, dietary supplementation of brewers' yeast showed significant effect on weight gain ($Y = 8.61x + 42.2$, $r^2 = 0.9495$) and survival ($Y = 7.252x + 59.07$, $r^2 = 0.9995$). Control group showed lowest growth when compared with treatment groups. Our

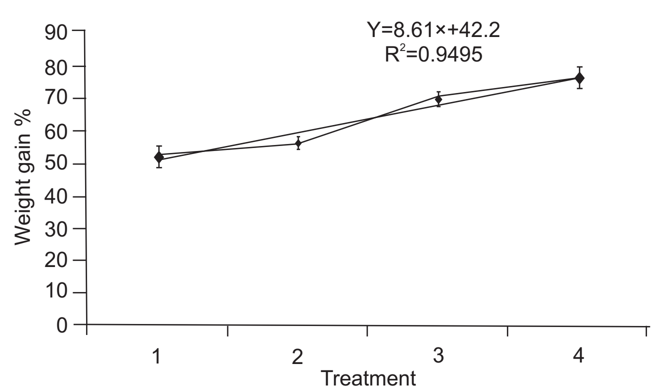

Fig. 1. Weight gain% of *Labeo rohita* fed diets containing graded levels of brewer's yeast for 120 days in a feeding trial.

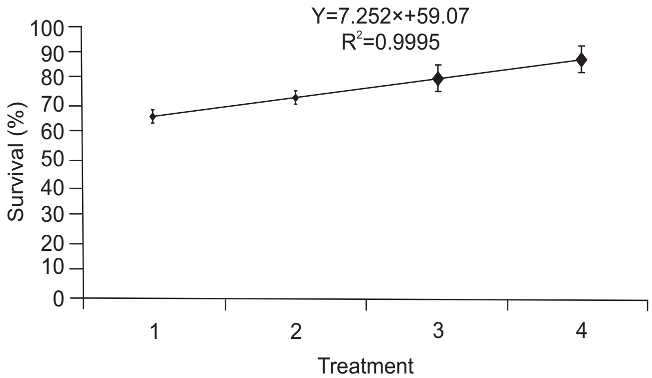

Fig. 2. Survival % of *Labeo rohita* fed diets containing graded levels of brewer's yeast for 120 days in a feeding trial.

results were supported by the findings of Li *et al*. (2005), who reported that dietary supplementation of brewers' yeast, especially at 1%, improved the growth and health of fish in hybrid striped bass (*Morone chrysops* × *M. saxatilis*).

Brewer's yeast is included in commercial diet formulations for several fish species, including salmonids (National Research Council 1993). The cell walls of yeast provide very important non-nutritive compounds that may benefit fish health, including mannose polymers covalently linked to peptides (mannoprotein), glucose polymers (glucans), chitin in minor amounts (Cabib *et al*. 1982) as well as nucleic acids (Rumsey *et al*. 1991).

Effect of brewer's yeast on immune responses

Nitro blue tetrazolium assay and Lysozyme activity of fish fed brewer's yeast incorporated was significantly higher ($P<0.05$) than that of control diet and Lysozyme activity showed no significant between and control group (Table 2). This is supported by the findings of Li and Gatlin III (2003) who indicated significant difference among 1% and 2% of brewer's yeast with that of basal diets.

Brewer's yeast is a source of nucleic acids and polysaccharides including β-1,3 glucans and has been recognized to effectively enhance immune functions of many aquaculture species including African catfish, Atlantic salmon (Engstad *et al*. 1992), rainbow trout (Jorgensen *et al*. 1993, Siwicki *et al*. 1994) and shrimp, *Penaeus monodon* (Thanardkit *et al.* 2002). β -1, 3-glucan is generally viewed as the factor in brewer's yeast with a known immunological mechanism (Gannam and Schrock 2001). Sakai *et al*. (2001) reported that the nucleotides from brewer's yeast and RNA were capable of enhancing the phagocytic and oxidative activities of kidney phagocytic cells, serum lysozyme in common carp as well as resistance to *Aeromonas hydrophila*.

In the present study no significant difference in the serum lysozyme activity of fish fed with brewer's yeast was observed among treatment and control groups. The results are comparable to previously reported studies that the fish which were orally administered glucan or chitin did not show increased serum lysozyme activity (Verlhac *et al*. 1998, Esteban *et al*. 2001, Ortuno *et al*. 2002).

Table 2. Effect of brewer's yeast on Nitro blue tetrazolium assay and Lysozyme activity after feeding trial

Treatments	Control	T_1	T_2	T_3
Nitro blue tetrazolium assay	0.523^a	0.624^a	1.142^b	1.698^c
Lysozyme activity	469.00^a	471.33^a	472.66^{ab}	473.09^b

Different superscripts (abc) in the same row indicate significant difference ($P<0.05$) among control and treatment groups (C, T_1, T_2 and T_3); Duncan's multiple range test $P\leq0.05$; The value expressed as a mean ± SE (n,6).

Effect on infection of Aeromonas hydrophila infection

In our investigation, control group showed higher mortality compared to treatment group to the relative percent survival was significantly higher in the treatment groups fed with graded levels of brewer's yeast and the control group. Single cell protein, including bacteria, have been viewed as promising substitutes for fishmeal in fish diets. Nutritional value of brewer's yeast *S. cerevisia* was evaluated in lake trout (Rumsey *et al*. 1990 and 1991), in rainbow trout and sea bass, *Dicentrarchus labrax* (Oliva-Teles and Goncalves 2001) by studying growth performance, feed efficiency and nitrogen retention. In recent years, several reports indicated that oral administration of nucleotides enhanced immune responses and/or disease resistance in several fish species; Burrells *et al*. (2001), Sakai *et al*. (2001), and Swain *et al*. (1996) recorded the better performance of mrigal, *Cirrhinus mrigala*, fry in terms of growth and disease resistance when fed diet supplemented with 0.15% of a probiotic.

The disease challenge against *Aeromonas hydrophila* infection resulted in 66.67% mortality of fish fed the control diet. The relative percent survival of fish fed diet containing brewer's yeast in T_3 (76.18%) was significantly ($P<0.05$) higher. Survival rate increased with graded supplementation of brewer's yeast in the diets (Table 3).

The overall outcome of the present investigation suggested that the inclusion of brewer's yeast @ 3 g/kg in diet, will

Table 3. Mortality and relative percent survival of *Labeo rohita* against *Aeromonas hydrophila*

Treatment	% mortality	RPS (%)
Control	66.67^d	33.33^a
T_1	42.86^c	57.14^{bc}
T_2	38.10^{bc}	61.90^c
T_3	23.82^a	76.18^d

Different superscripts (abcd) in the same row indicate significant difference ($P<0.05$) among control and treatment groups (C, T_1, T_2 and T_3); Duncan's multiple range test a, 0.05; the value expressed as a mean ± SE (n,6).

improve growth performance and enhance the immune response as well as the relative per cent survival of *Labeo rohita* fingerlings. These results may be useful for the farmers to improve the fish production per unit area.

REFERENCES

Amend D F. 1981. Potency testing of fish vaccines-Serodiagnostics and Vaccines Development and Biological Standard. *International Symposium on Fish Biologics:* 447–54.

Anderson D P, Siwicki A K and Rumsey G L**.** 1995. Injection or immersion delivery of selected immunostimulants to trout demonstrate enhancement of nonspeciûc defense mechanisms and protective immunity. *Diseases in Asian Aquaculture.* vol. 11. pp. 413–26. (Eds) Shariû M, Subasighe R P and Arthur J R. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Blaut M. 2002. Relationship of prebiotics and food to intestinal microflora. *European Journal of Nutrition* **41**: 11S–16S.

Burrells C, William P D and Forno P F. 2001. Dietary nucleotides: a novel supplement in fish feeds. Effects on resistance to diseases in salmonids. *Aquaculture* **199**: 159–69.

Cabib E, Roberts R and Bower B. 1982. Synthesis of the yeast cell wall and its regulation. *Annual Review of Biochemistry* **51**: 763–93.

Ellis A E, Stapleton K J and Hastings T S. 1988. Thehumoral immune response of rainbow trout (*Salmo gairdnen*) immunised by various regimes and preparations of *Aeromonas salmonicida* antigens *Veterinary Immunology and Immunopathology* **119**: 153–64.

Engstad R E, Robertsen B and Fivlod E. 1992. Yeast glucan induces increase in lysozyme and complement mediated haemolytic activity in atlantic salmon blood. *Fish and Shellfish Immunology* **2**: 287–97.

Esteban M A, Cuesta A, Ortuno J and Meseguer J. 2001. Immunomodulatorr effect of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish Shellfish Immunology* **11**: 303–15.

FAO (Food and Agriculture Organisation). 2006. *Fisheries Statistics - Catch and landing (2004)* Vol. **80**. FAO Fisheries Series no. 41. FAO of United Nation, Rome.

Gannam A L and Schrock R M. 2001. Immunostimulants in fish diets. *Nutrition and Fish Health.* pp. 235–66. (Eds) Lim C, Webster C D. Haworth Press, Binghamton, USA.

Jorgensen J B, Sharp G J E, Secobes C J and B Robertsen. 1993. Effect of yeast cell wall glucan on bactericidal activity of rainbow trout macrophages. *Journal of Fish Biology* **31**(A): 51–56.

Li P and D M Gatlin III. 2003. Evaluation of brewer's yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops_M. saxatilis*). *Aquaculture* **219**: 681–92.

Li P, Lewis D H and D M and Gatlin III. 2005. Evaluation of the prebiotic, GroBiotic®-A and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture* **257** (1–4) : 393–99.

Lillehaug. 1989. A cost-effective study of three different methods of vaccination against vibriosis in salmonids. *Aquaculture* **83**: 227–36.

Muiswinkel W B, Van Anderson, Lamers D P, Egberrs C H J, Loon E, Van J J A and Ijssel J P. 1985. Fish immunology and fish health. *Fish Immunology.* pp. 1–8. (Ed.) Manning M J. Academic press London.

Murthy H S. 2002. Indian major carps. *Nutrient requirements of fish for aquaculture* (Eds.) Wenster C D. and Lim C. CAB International, U K.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. Pp.114. National Academy Press, Washington, D C, USA.

Oliva-Teles A and P Goncalves. 2001. Partial replacement of fishmeal by brewer's yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **202**: 269–78.

Ortuno J, Cuesta A, Rodrigues A, Esteban M A and Meseguer J J. 2002. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). *Veterinary Immunology and Immunopathology* **85**: 41–50.

Raa J, Rorstad G, Engstad R and Roberisen B. 1992. The use of immunostimulants to increase resistance of aquatic organismsto microbial infections. *Diseases in Asian Aquaculture* **1**: 39–50.

Rao K G, Mohan C V and Seenappa D. 1992. The use of chemotherapeutic agents in fish culture in India. *Diseases in Asian Aquaculture.* Pp. 505–14. (Eds) Shariff I M, Sbasinghe R P and Arthur J R. Fish health section, Asian fisheries society, Manila, Philippines.

Rumsey G L, Hughes S G and Kinsella J L. 1990. Use of dietary yeast *Saccharomyces cerevisiae* nitrogen by lake trout. *Aquaculture* **21**: 205–09.

Rumsey G L, Kinsella J E, Shetty K J, and Hughes S G. 1991. Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout. *Animal Feed Science and Technol*ogy **33**: 177–83.

Sakai M, Taniguchi K, Mamoto K, Ogawa H and Tabata M. 2001. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio L. Journal of Fish Diseases* **24**: 433–38.

Siwicki A K, Anderson D P and Rumsey G L. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology* **41**: 125–39.

Swain S K, Rangacharyulu P V, Sarkar S and Das K M. 1996. Effect of probiotic supplement on growth, nutrient utlisation and carcass composition in mrigal fry. *Journal of Aquaculture* **4**: 29–35.

Thanardkit P, Khunrae P, Suphantharika M and Verduyn1 C. 2002. Glucan from spent brewer's yeast: preparation, analysis and use as a potential immunostimulant in shrimp feed. *World Journal of Microbiology and Biotechnology* **18**: 527–39.

Van Muiswinkel W B and Wigertjes G F. 1997. Immune responses after injection, vaccination of fish. *Fish vaccinology, Development of Biological Standards* **90**: 55–57. (Eds) Gudding, R, Lillehaug A, Midtlying P J and Brown F. Basel Karger.

Verlhac M H, Lefebvre C, Kubiak J Z and Umbhauer M. 1998. Mos activates MAP kinase in mouse oocytes through two opposite pathways. *Journal EMBO* **19**: 6065–74.