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FEED SUPPLEMENTATION WITH MARINE YEAST *CANDIDA* MCCF 101 ENHANCES FISH HEALTH: ASSESSED THROUGH HAEMATOLOGICAL PARAMETERS

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Abstract: Faster growth and disease resistance of the cultured species are the most important concerns in present aquaculture scenario. Farmers emphasize on diagnosis and prevention of infection to promote health and productivity. Increased concern about antibiotic resistant microorganisms has led to the use of alternative dietary supplements such as probiotics and prebiotics to enhance the health and production of cultured fish. In recent years there has been heightened research in developing dietary supplementation strategies in which various health-promoting compounds have been evaluated. In this context blood parameters are useful and sensitive assays for enhanced non – specific immune system and monitoring of the physiological status of fish. Therefore this study was undertaken to determine the health of ornamental fish, Koi carp, fed on the marine yeast *Candida* MCCF 101 by analysis of a few hematological parameters. The hematological parameters such as Hb, RBC, WBC, PCV, Glucose, albumin, globulin and protein were considered for analyzing their protective role. The results revealed that the marine yeast *Candida* MCCF 101 fed fishes exhibited higher RBCs (1.14 ± 0.20 10^6/µL), Hb (7.83 ± 0.20g dL^{-1}), PCV (25.76 ± 2.86), glucose (82 ± 16.64 mg/dL), albumin (2.51±0.14 g dL^{-1}) , globulin (1 ± 0.50 g /dL) and protein values (2.83 ± 0.29(g/dL) than those of control. The study demonstrates that supplementation of marine yeast *Candia* MCCF 101 has a positive influence on the health the fish, Koi carp.

Key words: Marine yeast, Fish health management, Blood indices, Immune system, Koi carp

INTRODUCTION

Rapid growth and disease resistance are the most important concerns in present aquaculture scenario. Intensive aqua farming accompanies several diseases often due to opportunistic pathogens. High stocking density, high feed inputs and other organic load stimulate the selection and proliferation of opportunistic pathogens (Austin *et al*., 1995). However, with changing scenario farmers are emphasizing on diagnosis and prevention of infection to promote health and production efficiency. The fish health management has now become an integral part of ornamental fish quality assurance programme (Abraham *et al*., 2008). Though the use of antibiotics and chemotherapy remains the method of choice as disease control strategy, the abuse of chemotherapeutics, especially antibiotics has resulted in the development of multiple antibiotic resistant bacteria (Alderman and Hastings, 1998; Teuber, 2001). Increased concern about antibiotic resistant microorganisms has led to the use of alternative dietary supplements such as probiotics and prebiotics to enhance the health and production of cultured fish (Verschuere *et al*., 2000; Merrifield *et al*., 2010; Ringo *et al*., 2010). In recent years there has been heightened research in developing dietary supplementation strategies in which various health-promoting compounds have been evaluated. Yeast single-cell proteins (SCPs) are playing a greater role in the evolution of aquaculture diets. Some yeast, like *Candida* sp. and *Saccharomyces cerevisiae*, are believed to have immunostimulatory properties by virtue of their complex carbohydrate components and nucleic acid content (Anderson *et al*., 1995). With excellent nutrient profiles and capacity to be mass produced economically, SCPs have been added to aquaculture diets as partial replacement of fishmeal (Lim *et al*., 2005). Yeast contains various immunostimulating compounds such as â-glucans, nucleic acids as well as mannan oligosaccharides, and it has the capability to

enhance immune responses (Ortuno *et al*., 2002) as well as growth (Li and Gatlin III, 2005) of various fish species. Marine yeasts have been regarded as safe and showing beneficial impact on biotechnological process. It provides better nutritional and dietary values indicating their potential application as feed supplements in aquaculture. Zhenming *et al*. (2006) reported still a lack of feed composed of single cell protein (SCP) from marine yeasts with high content of protein and other nutrients. The dietary intake of whole yeast cells leads to enhanced phagocytosis and respiratory burst (Cuesta et al., 2007). In this context blood parameters are regarded as valuable tools for assessing the immune system and monitoring of the physiological status of fish health (Adhikari *et al*., 2004). Therefore this study was undertaken to determine health of the ornamental fish, Koi carp, fed on the marine yeast *Candida* MCCF 101 by way of analysis of a few hematological parameters.

MATERIALS AND METHODS

Preparation of yeast biomass

Yeast biomass was generated in pilot scale fermentor containing mineral based medium (maltose -50.8 gL^{-1}, $MgSO_4.7H_2O$-1.8 gL^{-1}, and yeast extract-18 gL^{-1}) with pH 6.51 and incubated at 26.3°C for 72 hrs.

Experimental diet

Experimental diets were prepared by incorporating yeast biomass to standard fish diet. This was done initially by incorporating yeast to the binder, 'Stick On – India' and coating on to the feed. After air drying for few hours, the pellets were kept in desiccator over silica gel overnight (12 hrs).

Experimental Animals

Koi carp (*Cyprinus carpio haematopterus*), the fresh water ornamental fish, obtained from a fish hatchery located at Thrissur, Kerala, India was used for the study. Fishes weighing ~1g ± 0.2g were acclimatized in a covered aquarium tank containing fresh water over a period of two weeks until feed consumption and general behavior became normal. Water temperature ranged from 27° to 29°C, dissolved oxygen concentrations from 4.3 to 6.7 mgL^{-1}, pH from 7.2 to 8.0, and unionized ammonia concentration from 0.04 to 0.14 mgL^{-1}. After the period of acclimatization, the fishes were transferred to the experimental tanks and were allowed to acclimatize for another week.

Experimental design

After acclimatization, fishes were randomly divided in to two groups. One was kept as control and the other group as test fed on a diet supplemented with marine yeast *Candida* MCCF 101. Each group consisted of three replicates of 10 animals in each tank, i.e., n= 30. The basic physico-chemical parameters of water viz. temperature, dissolved oxygen, NH_3-N, NO_2-N and NO_3-N were monitored daily following standard procedures (APHA, 1995) and maintained at optimal levels. Stringed Bed Suspended Bioreactor (SBSBR) (Kumar *et al*., 2009) was maintained in all the tanks to manage ammonia level around 0.1ppm.

Feeding regime

Control groups were fed on commercial feed without supplementation of yeast. Remaining groups were fed with yeast. The yeast incorporated diets were initially fed at 10% of body weight for 4 weeks, subsequently reduced to 5% during the remaining weeks. Each diet was fed twice daily for a period of three months. Uneaten pellets were siphoned out of the tanks.

Haematological parameters

Fishes were not fed for 24 hrs prior to blood sampling and were anaesthetized with clove oil in ethanol at a ratio of 1:10 (v/v) and added to water to get a final strength of 80 ppm. The point at which the fishes lost sensitivity to touch was used for blood collection. Blood was collected by tail ablation. Using a haematocrit tube, blood was taken from the caudal vein and divided in to two sets of Eppendorf tubes. One set contained a pinch of EDTA (ethylene diamine tetraacetic acid) used as an anticoagulant for haematological analysis (Hb, RBC, WBC and PCV). The second set was left to clot at 4°C and centrifuged at 5000 rpm for 5 min at room temperature. The collected serum was stored at -20°C for further assays (glucose, albumin, globulin and protein). Blood samples pooled from a random sample of fishes in each experimental tank was used.

Haemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanmethaemoglobin using a commercial kit. In this method the ferrous ion (Fe^{2+}) of haemoglobin is oxidized to ferric state (Fe^{3+}) by potassium ferricyanide to form methaemoglobin. The methaemoglobin then reacts with cyanide ions from potassium cyanide to form cyanmethaemoglobin which is measured colorimetrically. Red blood cells (RBCs) and White blood cells (WBC) were counted under a light microscope using a Neubauer haemocytometer following the method described by Praful and Darshan (2003). Packed cell volume (PCV) was determined by microhaematocrit. Micro haematocrit employs small capillary tube of 8 cm length with a uniform pore size of 1 mm diameter. PCV as cell volume percentage was measured directly on a microhaematocrit reader associated with the centrifuge. Glucose was determined colorimetrically according to Sasaki *et al*. (1972). Serum total protein content was estimated by the method of Lowry *et al*. (1951). Total lipid content was determined colorimetrically according to Barnes and Blackstock (1973). Albumin and globulin were determined colorimetrically following Bartholomew *et al*. (1966).

RESULTS

Haematological parameters

Haematological parameters are shown in Table 1. Mean values having the same superscript in the same row are not significantly different at P<0.05.

Haemoglobin content was significantly (p d" 0.05) higher as compared to control in the batches of fishes fed on yeast (7.83 ± 0.20 gdL^{-1}). However, WBC count was significantly higher (p< 0.05) in the control group (666 ± 230.9 × 10^3 μL^{-1}). RBC count (1.14 ± 0.20 $10^6/\mu L$) PCV (25.76 ± 2.86 %) and Hb content were higher in the test group (p<0.05). A significantly higher blood glucose level was found in the group of fishes fed with yeast (82 ± 16.64 mg dL^{-1}) compared to that of the control (48.3 ± 6.66 mg dL^{-1}). Serum protein level registered slight increase in the batches of fishes fed on yeast (2.83 ± 0.29g dL^{-1}) compared to the one recorded in the control (2.63 ± 0.38 g dL^{-1}). Albumin levels were positively influenced by *Candida* MCCF 101 supplement, the highest in the fishes fed with the yeast (2.51±0.14 g dL^{-1}). Globulin levels did not significantly (P>0.05) differ between the treatment groups (1 ± 0.50 g/dL^{-1} and 0.8 ± 0.60 g/dL^{-1} respectively, however, was higher in the test animals).

DISCUSSION

Hematological analyses often provide vital information for health assessment and management of cultured fish (Rehulka *et al*., 2004). In the present study it could be demonstrated that yeast supplementation exerted a certain level of influence on some of the blood parameters in favour of the animal. They were increase in HB, RBC, PCV, glucose, albumin, globulin and protein. Among them the increase of HB and glucose levels in blood of the yeast fed group was statistically significant. The increase of Hb content points to the fact that yeast supplementation in feed plays a significant

Table 1. Haematological parameters of Koi carp fed with *Candida* MCCF 101

Items	Control	*Candida* MCCF 101
HB (g/dl^{-1})	4.8 ± 0.61^b	7.83 ± 0.20^a
RBC ($10^6/\mu L^{-1}$)	0.72 ± 0.18^a	1.14 ± 0.20^a
WBC ($10^3/\mu L^{-1}$)	666 ± 230.9^a	333.35 ± 7.7^a
PCV (%)	14 ± 2.80^a	25.76 ± 2.86^a
Glucose (mg/dL^{-1})	48.3 ± 6.66^b	82 ± 16.64^a
Albumin (g/dL^{-1})	1.84 ± 0.29^a	2.51±0.14 ^{a}
Globulin (g/dL^{-1})	0.8 ± 0.60^a	1 ± 0.50^a
Protein (g/dL^{-1})	2.63 ± 0.38^a	2.83 ± 0.29^a

role in iron absorption and mobilization to the production of more RBCs (Grimble, 1996). Heightened blood glucose level indicates enhancement of defense to pathogenic invasion. Increase in the serum protein, albumin and globulin is associated with a stronger innate response in fishes (Jha *et al*., 2007). Moreover, the measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value, as it relates to general nutritional status as well as the integrity of the vascular system and liver function.

In conclusion, feeding Koi carp with the marine yeast, *Candida* MCCF 101, at moderate level ($1x10^{6}$ g^{-1}), feed improves over all health of the animal.

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