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Study of Haematological Parameters in Catfish *Pangasianodon hypophthalmus* Fed with Probiotics Supplemented Diets

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Abstract: ***The effect of probiotics as dietary supplement on haematological parameters of the catfish Pangasianodon hypophthalmus was studied under laboratory conditions. The fishes were fed for 60 days with two different diets supplemented with bacterial suspension of Bacillus subtilis and Saccharomyces cerevisiae respectively at x10^8 CFU g^{-1}and control group was maintained without the probiotic. Blood samples were collected at the intervals of 15, 30, 45 and 60 days. The haematological parameters such as total erythrocyte count (RBC), total leucocyte count (WBC), haematocrit (Hct), haemoglobin (Hb) and haematological indices (MCV, MCH and MCHC) were examined. Haematological parameters showed elevated levels in fishes fed with probiotic Bacillus subtilis supplemented diet. The results indicate that Bacillus subtilis proves to be a better probiotic tool than Saccharomyces cerevisiae which may be preferred for aquaculture practices.***

Keywords: Probiotic, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Pangasianodon hypophthalmus*, Haematological parameters

1. Introduction

Pangasianodon hypophthalmus is an aquaculture fish with relatively high growth rate. Many disease outbreaks affecting fish survival and its growth in aquaculture industry have been reported [19]. Use of probiotic bacteria in aquaculture is one of the new approaches that is gaining widespread acceptance to control potential pathogens [8, 23]. Probiotics can be defined as a live microbial feed supplement that beneficially affects the host by improving the intestinal microbial balance. Growth promotion or protection of fish against bacterial pathogens can be achieved by selecting bacterial strains which have the capacity to colonize the fish by adhesion and to increase the digestive performance of fish by producing vitamins. *Bacillus licheniformes* as dietary supplementation significantly increases growth rate in prawn and shrimp [13, 25]. *Bacillus subtilis* is a Gram-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. *B. subtilis* is rod-shaped and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. Bacillus spores are being used as probiotic due to their immunostimulatory properties on the gastrointestinal immune system [4]. Bacillus secretes many enzymes that by degrading slime and biofilms allow their antibiotics to penetrate slime layer around Gram negative bacteria. *Saccharomyces cerevisiae* is the most common yeast species, which produces energy substrates for intestinal cells; thereby contributes to the healthy gut. The cell wall extracts of *S. cerevisiae* (mannoprotein, glucan, and chitin) are valuable, natural immunostimulants [7].

The present investigation was undertaken to study the effect of probiotics *Bacillus subtilis* and *Saccharomyces cerevisiae* supplemented diets on the haematological parameters in catfish *Pangasianodon hypophthalmus*. Haematological parameters help to understand the immune response to disease resistance in the fish along with enhanced healthy conditions.

2. Materials and Methods

The fishes were brought and acclimatized under laboratory conditions for a period of one week in well aerated glass aquaria. The fishes were distributed in three groups namely Group 1 (*B. subtilis* diet), Group 2 (*S. cerevisiae* diet) and Group 3 (control diet with no probiotic). The base diet included ingredients such as fish meal, soya meal, rice polish, wheat bran, corn flour, sunflower oil, cod liver oil, vitamin+mineral mix, vitamin C, vitamin B-complex, carboxymethyl cellulose (CMC), butylated hydroxytoluene (BHT) and glycine. The probiotics were added to the base diet at x10^8 CFU g^{-1}, fed twice a day in proportion of 4% body weight of the experimental fishes. After an interval of two days the water in the aquaria was changed to remove the faecal matter. The water temperature varied between 26^{o}C to 29^{o}C and pH 7.2 maintained throughout the experimental period.

Blood samples were collected in EDTA (anticoagulant) tubes from the fish heart using 2 ml EDTA rinsed syringes at the interval of 15, 30, 45 and 60 days. Haematological parameters such as total erythrocyte (RBC) and total leucocyte (WBC) counts were determined by using improved Neubauer haemocytometer. Haemoglobin (Hb) concentration was estimated by cyanmethemoglobin method using Drabkin's reagent (Biolab Diagnostics) and haematocrit value (Hct) was calculated. Using Dacie and Lewis calculations, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were determined.

3. Statistical Analysis

One way analysis of variance (ANOVA) and statistical assessment of result was carried out using SSPS software 16 version.

4. Result and Discussion

Haematological parameters of *Pangasianodon hypophthalmus* studied for the period of 15, 30, 45 and 60 days respectively are shown in Tables 1, 2 and 3.

Table 1: Haematological parameters of *Pangasianodon hypophthalmus* fed at 15, 30, 45 and 60 days with diet without probiotic.

Haematological parameters	Days				
	Initial	15	30	45	60
RBC (x $10^6/\mu l^{-1}$)	2.28 ± 0.04	2.35 ± 0.04	2.51 ± 0.06	2.74 ± 0.06	3.02 ± 0.04
WBC (x $10^3/\mu l^{-1}$)	4.93 ± 0.6	4.98 ± 0.4	5.20 ± 0.53	5.33 ± 0.83	5.40 ± 0.53
Hb (g/dL)	3.97 ± 0.42	4.46 ± 1.05	5.12 ± 0.42	5.73 ± 0.42	6.20 ± 0.64
Hct (%)	10.97 ± 1.12	12.84 ± 2.83	15.47 ± 1.13	16.60 ± 1.13	17.47 ± 1.72
MCV (fL)	44.86 ± 1.00	51.14 ± 1.39	53.95 ± 0.24	55.76 ± 2.55	56.79 ± 1.66
MCH (pg)	15.40 ± 0.80	17.77 ± 1.05	18.36 ± 1.38	19.40 ± 0.68	20.15 ± 0.77
MCHC (g/dL)	34.32 ± 0.30	34.66 ± 0.59	35.06 ± 0.15	35.29 ± 0.13	35.71 ± 0.13

Table 2: Haematological parameters of *Pangasianodon hypophthalmus* fed at 15, 30, 45 and 60 days with probiotic *Bacillus subtilis* supplemented base diet.

Haematological parameters	Days				
	Initial	15	30	45	60
RBC (x $10^6/\mu l^{-1}$)	2.28 ± 0.04	2.78 ± 0.04	3.12 ± 0.06	3.46 ± 0.06	3.92 ± 0.04
WBC (x $10^3/\mu l^{-1}$)	4.93 ± 0.6	5.41 ± 0.4	5.74 ± 0.53	6.02 ± 0.83	6.37 ± 0.53
Hb (g/dL)	3.97 ± 0.42	5.36 ± 1.05	6.07 ± 0.42	6.74 ± 0.42	7.78 ± 0.64
Hct (%)	10.97 ± 1.12	15.26 ± 2.83	17.17 ± 1.13	18.97 ± 1.13	21.77 ± 1.72
MCV (fL)	44.86 ± 1.00	54.87 ± 1.39	57.03 ± 0.24	59.54 ± 2.55	62.29 ± 1.66
MCH (pg)	15.40 ± 0.80	19.28 ± 1.05	20.85 ± 1.38	21.48 ± 0.68	22.65 ± 0.77
MCHC (g/dL)	34.32 ± 0.30	35.12 ± 0.59	35.85 ± 0.15	36.53 ± 0.13	37.06 ± 0.13

Table 3: Haematological parameters of *Pangasianodon hypophthalmus* fed at 15, 30, 45 and 60 days with probiotic *Saccharomyces cerevisiae* supplemented base diet.

Haematological parameters	Days				
	Initial	15	30	45	60
RBC (x $10^6/\mu l^{-1}$)	2.28 ± 0.04	2.48 ± 0.04	2.68 ± 0.06	2.97 ± 0.06	3.35 ± 0.04
WBC (x $10^3/\mu l^{-1}$)	4.93 ± 0.6	5.22 ± 0.4	5.36 ± 0.53	5.61 ± 0.83	5.78 ± 0.53
Hb (g/dL)	3.97 ± 0.42	4.98 ± 1.05	5.39 ± 0.42	6.15 ± 0.42	6.88 ± 0.64
Hct (%)	10.97 ± 1.12	14.23 ± 2.83	16.34 ± 1.13	17.73 ± 1.13	19.38 ± 1.72
MCV (fL)	44.86 ± 1.00	52.88 ± 1.39	54.65 ± 0.24	56.91 ± 2.55	59.05 ± 1.66
MCH (pg)	15.40 ± 0.80	18.36 ± 1.05	19.26 ± 1.38	20.11 ± 0.68	21.32 ± 0.77
MCHC (g/dL)	34.32 ± 0.30	34.99 ± 0.59	35.32 ± 0.15	35.98 ± 0.13	36.69 ± 0.13

RBC- Red Blood Cell count, WBC- White Blood Cell count, Hb- Haemoglobin, Hct- Haematocrit value,

MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration
p values: RBC (0.23), WBC (0.16), Hb (0.51), Hct (0.62), MCV (0.63), MCH (0.50), MCHC (0.41)

Fish under intensive culture conditions are badly affected and often fall prey to different microbial pathogens. Chemotherapeutic substances as antibiotics are often used as a curative measure. Probiotics and their products which provide health benefits have been used as one of the alternative to reduce the effects of chemotherapeutic substances [19]. Probiotics are considered as natural immunostimulants which are biocompatible, biodegradable, safe for environment as well as human health, adding to the nutritional value of the organism [10].

According to Marzouk et al., 2008, *B. subtilis* and *S. cerevisiae* are frequently used probiotics which adhere and colonize the *O. niloticus* gut, preventing the adhesion and colonization of specific fish pathogens. β-glucan and chitin secreted by *S. cerevisiae* have been described as powerful immunostimulants in fish and mammals. They stimulate the cellular and humeral non-specific defence of fish against diseases [20, 26]. Kumar et al., 2014, reported increased levels in the haematological parameters of climbing pearch, *Anabas testudineus* fed with *Bacillus licheniformis.* All the haematological parameters showed increased levels in *P. hypophthalmus* fed with *B. subtilis* as compared to *S. cerevisiae* in the present study. Further, *Bacillus subtilis* supplementation in diet may help to enhance immune response thereby increase disease resistance in the fishes. This is in agreement with work reported by Ziemer and Gibson, 1998. Jessus Ortuno et al., 2002, reported that oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream. The present investigation suggests that probiotics *B. subtilis* and *S. cerevisiae* may be used in supplementing fish diet to increase disease resistance and provides a control strategy in aquaculture to curb mass mortalities due to pathogens.

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References

[1] Anjni Kumari, Ajit Kumar, Shakti Kumar (2014). Increase in growth and haematological parameters by *Bacillus licheniformis* dietary supplementation to climbing perch, *Anabas testudineus* (Bloch, 1792). International Journal of Fisheries and Aquatic Studies. 2(2): 215-219.

[2] Aoki T, Kanazawa T, and Kitao T (1985). Epidemiological surveillance of drug-resistant *Vibrio anguillarum* strains. Fish Patho 20: 199-208.

[3] B. Gohila, Dr. R. Damodaran, V. Bharathidasan and M. Vinoth (2013). Comparative Studies on Growth Performance of Probiotic Supplemented Rohu (*Labeo rohita*) Fingerlings. International Journal of Pharmaceutical & Biological Archives 4(1): 84-88.

[4] Casula G, Cutting SM (2002). *Bacillus* probiotics: spore germination in the gastrointestinal tract. App EnvironMicrobiol 68:2344-2352.

[5] Cerezuela R, Guardiola FA, Meseguer J, Esteban M (2012). Increases in immune parameters by inulin and *Bacillus subtilis* dietary administration to gilthead seabream (*Sparusaurata* L.) did not correlate with disease resistance to *Photobacterium damselae*. Fish & shellfish immunology 32(6):1032-1040.

[6] Dacie JV, Lewis SM. Practical haematology, 9th edition (2001). Churchill Livingstone, London 663.

[7] Esteban MA, Rodriguez A, Mesguer J (2004). Glucan receptor but not mannose receptor is involved in the phagocytosis of Saccharomyces cerevisiae by seabream (Sparusauratus L.) blood leucocytes. Fish Shellfish Immunol 16:447–51.

[8] Gomez-Gil B., Roque A. and Turnbull J. F (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquac 191: 259-270.

[9] Gurusamy Chelladurai1, Jebaraj Felicitta1, Rathinasami Nagarajan (2013). Protective effect of probiotic diets on haematobiochemical and histopathology changes of *Mystus montanus* (Jerdon 1849) against *Aeromonas hydrophila*. Journal of Coastal Life Medicine doi:10.12980/JCLM.1.c1088.

[10] Jessus Ortuno, Alberto Cuesta, Alejandro Rodriguez, M. Angeles Eesteban and Jose Meseguer (2002). Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gillhead seabream, Sparusaurata L. J. Veterinary immunology and immunopathology 85; 41-50.

[11] Joseluis Balcazar, Ignacio de Blas, Imanol Ruiz-Zarzuela, David Cunningham, Daniel Vendrell, Joseluis Muzquiz (2006). The role of probiotics in aquaculture Reviews. Veterinary Microbiology 114:173-186.

[12] Kritas SK, Govaris A, Christodoulopoulos G, Burriel AR (2006). Effect of *Bacillus licheniformis* and *Bacillus subtilis* supplementation of ewe's feed on sheep milk production and young lamb mortality. Journal of Veterinary Medicine Series A 53(4):170-173.

[13] Kumar NR, Raman RP, Jadhao SB, Brahmchari RK, Kumar K, Dash G (2013). Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquaculture International 21(2):387-403.

[14] Kumar R, Mukherjee SC, Prasad KP, Pal AK (2006). Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). Aquaculture Research 37:1215-1221

[15] Kumar R, Mukherjee SC, Ranjan R, Nayak SK (2008). Enhanced innate immune parameters in *Labeo rohita* (Ham.) following oral administration of *Bacillus subtilis*. Fish & shellfish immunology 24(2):168-172.

[16] Lakra, W.S. and Singh, A.K (2010). Risk analysis and sustainability of *Pangasianodon hypophthalmus* culture in India. National Bureau of Fish Genetic Resources, Uttar Pradesh India.

[17] Lazard, J. (1998). Interest of basic and applied research on *Pangasius* spp. for aquaculture in the Mekong Delta: situation and prospects. *In* M. Legendre & A. Pariselle, eds. The biological diversity in aquaculture of clariid and pangasiid catfish in South East Asia. Proceedings of the mid-term workshop of the "Catfish Asia Project", 11-15 May Can Tho, Viet Nam, pp. 15–20.

[18] M. Rajikkannu, Nirmala Natarajan, P. Santhanam, B. Deivasigamani, J. Ilamathi, S. Janani (2015). Effect of probiotics on the haematological parameters of Indian major carp (*Labeo rohita*). International Journal of Fisheries and Aquatic Studies 2(5): 105-109.

[19] Marzouk M.S, Moustafa M.M, Mohamed N.M (2008). The Influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. In: Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 1059-1071.

[20] Raa J (2000). The use of immune-stimulants in fish and shellfish feeds. International Symposium on Nutrition and Aquaculture, 19–22 November. Merida, Yucatan, Mexico.

[21] Rajesh Kumar Subhas C, Mukherjee Kurcheti Pani Prasad, Asim K Pal (2006). Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp, *Labeo rohita* Aquaculture Research 37:1215-1221.

[22] Raida MK, Larsen JL, Nielsen ME, Buchmann K (2003). Enhanced resistance of rainbow trout *Oncorhynchus mykiss* (Walbaum) against *Yersinia ruckeri* challenge following oral administration of *Bacillus subtilis and B. licheniformis* (Bio Plus 2B). Journal Fish Diseases 26:495-498.

[23] Robertson P. A. W., O'Dowd C., Burrells C., Williams P. and Austin B (2000). Use of carno bacterium sp. as probiotic for Atlantic salmon (*Salmosalar* L) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture 185: 235-243.

[24] S.O. Ayoola, E.K. Ajani and O.F. Fashae (2013). Effect of Probiotics (*Lactobacillus* and *Bifidobacterium*) on Growth Performance and Hematological Profile of *Clarias gariepinus* Juveniles, Department of Marine

Sciences, University of Lagos, Lagos State, Nigeria, Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria.

[25] Wang YB (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 269(1):259-264.

[26] Whittington R, Lim C, Klesius PH (2005). Effect of dietary b-glucan levels on the growth response and efficacy of Streptococcus iniae vaccine in Nile tilapia, O. niloticus. Aquaculture 248:217–25.

[27] Yassir A. E. Khattab, Adel M. E. Shalaby and Azza Abdel-Rhman. Use of probiotic bacteria as growth promoters, anti-bacterial and their effects on physiological parameters of *Oreochromis niloticus*. Journal of The Arabian Aquaculture Society.

[28] Zhou X, Tian Z, Wang Y, Li W (2010). Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. Fish physiology and biochemistry 36(3):501-509.

[29] Ziemer CJ, Gibson GR (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. International Dairy Journal 8(5-6):473-479.