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Effect of *Lactococcus Lactis*: a Probiotic tool, on Haematological Parameters in Catfish *Pangasianodon Hypophthalmus*

KEYWORDS: Probiotic, Lactococcus lactis, Pangasianodon hypophthalmus, haematological parameters

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ABSTRACT *Lactococcus lactis, a Gram-positive bacterium was mixed in the diet feed of the catfish Pangasianodon hypophthalmus. The fishes were fed with this diet for a period of sixty days. Blood samples were collected with an interval of 15 days to study the haematological parameters such as total erythrocyte (RBC) count, total leucocyte (WBC) count, haematocrit (Hct), haemoglobin concentration (Hb), and haematological indices (MCV, MCH and MCHC). All the haematological parameters showed elevated levels than those in control group suggesting that Lactococcus lactis could be used as an efficient probiotic tool for better aquaculture practices.*

INTRODUCTION

Currently, aquaculture is the fastest growing food producing sector in the world. *Pangasianodon hypophthalmus* is an aquaculture fish which can be easily processed and highly suited to fillet and yield around 35%. Their by-products are valuable for various applications, including fishmeal, bio-diesel, and cosmetics. The large-scale production from this sector is affected by outbreak of many infectious diseases (S. Vijayaram, 2014). The preventive measure taken is the use of antimicrobial drugs or chemotherapeutic substances. But, use of these substances often lead to the emergence of Antibiotic Resistant Bacteria (ARB) (Aditya Kesarcodi-Watson, 2008) affecting the aquaculture production and further causes a risk to human health. One excellent way to replace the much used antimicrobial drugs to prevent infectious microbial diseases is the use of probiotics in aquaculture. Probiotics serve the purpose of providing nutrition and prevention of pathogenic infection by developing immune system.

Lactic Acid Bacteria (LAB) are one of the potential probiotics in aquaculture and are also known to be present in the intestine of healthy fish. Proteinaceous bacteriocins produced by LAB strains serve as an added benefit to the fish as it reduces the pathogenicity of micro-organisms (Priyadarshini Pandiyan a, 2013). *Lactococcus lactis* is one of the most commonly used lactic acid bacterial strain in animal nutrition. The present study is undertaken to study the impact of *Lactococcus lactis* as a nutritional supplement in catfish *Pangasianodon hypophthalmus.*

Haematological characteristics help to monitor physiological changes in the fishes along with structural and functional health status. The haematological parameters serve as diagnostic tools to understand the pathophysiology of the whole fish body.

MATERIAL AND METHODS

The fishes were collected from fish farm and acclimatized for one week under laboratory conditions. The fishes were randomly distributed in triplicate groups in well aerated glass aquaria, each with 30 fishes, along with a control group and individual fish of approximately 10 to 15 gms in weight. The base diet included 11.4% fish meal, 42% soya meal, 13% rice polish, 11% wheat bran, 10% corn flour, 1% carboxymethyl cellulose (CMC), 8% sunflower oil: cod liver oil, 3% vitamin+mineral mix, 0.1% vitamin C, 1% vitamin B-complex, 0.2% butylated hydroxytoluene (BHT), 0.2% glycine. *Lactococcus lactis* at $x10^8$ CFU g^{-1} were added to the base diet and fed to experimental fishes twice a day at 2% body weight. Control group was maintained without the addition of the probiotic.

Blood samples were collected in EDTA (anticoagulant) tubes from the heart using 2 ml EDTA rinsed syringes at the interval of 0, 15, 30, 45 and 60 days. Total erythrocytes (RBC) and total leucocyte (WBC) counts were determined by using improved Neubauer haemocytometer. Haemoglobin (Hb) concentration was estimated by cyanmethemoglobin method, 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.

STATISTICAL ANALYSIS

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

RESULT

Haematological parameters of *Pangasianodon hypophthalmus* fed with base diet without the probiotic for sixty days is shown in Table 1, while Table 2 shows fishes fed with base diet and probiotic *Lactococcus lactis*

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Table 1: Haematological parameters of *Pangasianodon hypophthalmus* **fed with diet at different intervals (mean ± SD) without probiotic**

Haematological parameter	Days interval				
	Initial	15	30	45	60
RBC (x 10^6/μl^{-1})	2.45 ± 0.04	2.51 ± 0.04	2.78 ± 0.06	3.02 ± 0.06	3.25 ± 0.04
WBC (x 10^3/μl^{-1})	4.93 ± 0.6	5.00 ± 0.4	5.20 ± 0.53	5.33 ± 0.83	5.40 ± 0.53
Hb (g/dL)	3.77 ± 0.42	4.46 ± 1.05	5.44 ± 0.42	5.86 ± 0.42	6.55 ± 0.64
Hct (%)	10.97 ± 1.12	12.84 ± 2.83	15.47 ± 1.13	16.60 ± 1.13	18.47 ± 1.72
MCV (fL)	44.86 ± 1.00	51.14 ± 1.39	55.76 ± 0.24	54.95 ± 2.55	56.79 ± 1.66
MCH (pg)	15.40 ± 0.80	17.77 ± 1.05	19.36 ± 1.38	19.40 ± 0.68	20.15 ± 0.77
MCHC (g/dL)	34.32 ± 0.30	34.66 ± 0.59	35.16 ± 0.15	35.29 ± 0.13	35.48 ± 0.13

Table 2: Haematological parameters of *Pangasianodon hypophthalmus* **fed with diet at different intervals (mean ± SD) with probiotic**

Haematological parameter	Days interval				
	Initial	15	30	45	60
RBC (x 10^6/μl^{-1})	2.42 ± 0.04	2.52 ± 0.04	2.87 ± 0.07	3.18 ± 0.07	3.49 ± 0.08
WBC (x 10^3/μl^{-1})	4.87 ± 0.81	5.20 ± 0.60	5.40 ± 1.00	5.67 ± 0.61	5.93 ± 1.17
Hb (g/dL)	3.91 ± 1.05	5.16 ± 0.64	5.86 ± 0.42	6.55 ± 0.64	7.67 ± 0.64
Hct (%)	11.35 ± 2.83	14.72 ± 1.73	16.60 ± 1.13	18.47 ± 1.72	21.48 ± 1.73
MCV (fL)	46.83 ± 1.37	58.21 ± 1.53	57.55 ± 0.51	58.19 ± 1.01	61.51 ± 1.15
MCH (pg)	13.41 ± 0.20	16.97 ± 0.55	19.53 ± 1.34	22.34 ± 1.40	24.17 ± 0.78
MCHC (g/dL)	34.33 ± 0.72	35.04 ± 0.24	35.29 ± 0.13	35.47 ± 0.15	36.04 ± 0.69

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 (0.72), WBC (0.27), Hb (0.47), Hct (0.46), MCV (0.29), MCH (0.69), MCHC (0.49)

DISCUSSION

In recent times, population growth, worldwide poverty and degradation of environment are of major concern. Fish is a natural resource and is food for a large number of populations in the world. Fish under intensive culture conditions treated with chemotherapeutic substances of which antibiotics were intensively used will be badly affected and often fall prey to different microbial pathogens. These curative substances produce the problem of bacterial drug fastness on one hand and then public health hazards on the other hand (Robertson *et al.*, 2000). One of the alternatives is the use of natural immune stimulants in fish culture for the prevention of diseases is a promising new development and could solve the problems of massive antibiotic use. Natural immune stimulants are biocompatible, biodegradable and safe for both the environment and human health. Moreover, they possess an added nutritional value (Jessus *et al.*, 2002). The use of probiotic is disease bio control strategy in aquaculture as they improve the fish health and modify the fish associated microbial community (Gibson and Roberfroid, 1995).

Fish diet quality assessment can be analysed by haematological parameters. Highest values of Hb, RBCs and WBCs have been recorded by fish fed with probiotics supplemented diet (M. Rajikkannu et al., 2015). Similar results were obtained in the present study of catfish *Pangasianodon hypophthalmus* fed with probiotic *Lactococcus lactis.* The application of probiotics in aquaculture shows tremendous benefits of health in fishes without using antibiotics for growth and enhancing disease resistance. The application of probiotics will help to augment further growth in aquaculture industry.

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