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Effect of Probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) as Immune Stimulant on Hybrid Catfish *Heteroclarias*

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Authors' contributions

This work was carried out in collaboration between all authors. Author TAY designed the study and wrote the first draft of the manuscript. Author OAI performed the statistical analysis, author SMT wrote the protocol and author UPY managed literature searches. Authors TAY, OAI, SMT and UPY managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Study on the effect of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) as feed supplement and immune stimulant on hybrid *Heteroclarias* was carried out with 240 hybrid *Heteroclarias* fingerlings with mean weight 1.90 ±0.40 g fed probiotic supplement (GNC Ultra 25) for fifty six (56) days in 12 number 30 x 60 x 30 cm rectangular aquarium tanks. There were four treatments and each replicated three times. The inclusion level of probiotic fed supplement was at 0 g/kg, 1 g/kg, 2 g/kg and 3 g/kg. The experimental fish were fed at 3%,5%,7%, and 9% body weight at two weeks interval, The result showed that there was significant difference $p(<0.05)$ between control and other treatments in terms of specific growth rate and percentage survival. The microbial analysis showed that there was presence of Gram positive bacterial in the gut of the hybrid *Heteroclarias*, The treatments fed with probiotics have the higher plate count as compared to the control, the haematological analysis showed that blood parameters of control differed

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significantly p(<0.05) from other treatments. The inclusion levels of the probiotic supplement use in this study enhanced the growth and immune response of hybrid *Heteroclarias*, however inclusion level at 1 g/kg is sufficient to enhance the proliferation of the required bacteria.

Keywords: Heteroclarias; immune booster; fish feed (coppens); culture media and Lactobacillus acidophilus and Bifidobacterium bifidum.

1. INTRODUCTION

Probiotics are microbes which have a beneficial effect on their host by modifying the host ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, visa-viz the host response towards disease, and improving the quality of its ambient environment [1]. This implies that probiotics may include microbial additives that prevent pathogenic organism from proliferating or establishing in the gut, on the superficial structure and in culture environment of the cultured species that ensure optimal use of feed by aiding digestion, improve water quality and stimulate the immune system of the host [2]. They also stated that there is suggestive evidence that several probiotic strains such as *Lactobacillus acidophilus* are useful in boosting the immune response.

They are also selected pure culture isolates of one or more living microorganisms added to feed to proliferates in the host gastrointestinal (GI) tract [3]. They confer a healthy benefit on the host as significant microbial feed supplement in the field of prophylaxis [4]. The research on probiotics for animal health is increasing with the demand for environment-friendly microorganism. Some probiotics were designed to treat the rearing medium, like bio control when the treatment is antagonistic to pathogenic organism or bioremediation when water quality is improved to support the aquatic organism. Most probiotics have been studied by isolating and selecting strains from aquatic environment [5]. For example *Bifidobacterium* spp Gram positive, anaerobic, branched or pleomorphic rods can be isolated from a variety of materials such as human and animal faces, sewage and from the oral cavity [5]. There are wide range of micro algae, yeast (*Debaryomyces, Phaffia* and Saccharomyces) and Gram positive (*Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, lacobacillus, Streptcoccus, Weisslla*) and gram negative bacteria (*Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas and Vibrio*) that have been evaluated and known to be probiotics [5].

The use of antibiotic to treat or control disease in aquaculture have been reported to affect the fish intestinal microbiota [6,7] and cause prevalence of fish resistance to antibiotic [8,9]. This notorious adverse effect associated with the use of antibiotic in aquaculture has made European Union to ratify a ban for the use of all sub-therapeutic antibiotics as growth promoting agents or prophylaxes in animal production. There is an urgent need in aquaculture to look for alternative to antibiotic in disease control; therefore, probiotics seems to profer a solution and is increasingly viewed as an alternative to antibiotics in animal *production* and particularly aquaculture [10]. One of the common probiotics is *Lactobacillus and bifidobacterium sp* which are a Lactic Acid Bacteria (LAB) [11].

Although the idea of prebiotics and probiotics were used in animals for the purpose of enhancing growth and reducing early mortality [12]; in humans their potential nutritional advantages consist of preventive and curative effects against diseases, including intestinal dysfunctions, inflammatory bowel diseases and colon cancer amongst others [12,13]. In Nigeria apart from the problems of high cost of fish feeds and quality seed, disease outbreak is a major challenge in fish farming particularly in early life stages [14]. This study was carried out to evaluate the effect of *Lactobacillus and bifidobacterium* sp probiotics drug *GNC ultra* 25 as feed supplement when incorporated into fish feed and immune stimulant on hybrid *Hetetoclarias*. The choice of *Heteroclarias* in this study was due to its high market value, fast growth rate, improved hybrid vigour and resistant to disease amongst others.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at Toxicology unit, Fish Farm, Department of Water Resources, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, for 56 days.

2.2 Experimental Fish and Feed

Two hundred and forty (240) *Heteroclarias* fingerlings of average (Mean initial weight 1.9±0.4 g) were purchased from private commercial fish farm in Ilorin, Kwara State. The fish were acclimatized for 5 days and fed with commercial diet (0.6-0.8 mm size coppens). Twenty (20) fish samples were randomly selected and distributed in 12 aquarium tanks of 30 liters water holding capacity, and each of the tanks was filled with approximately 15 L of clean water. Four treatments were maintained and each treatment was replicated three times. The tested *ultra GNC* 25 probiotic drug (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) was added at inclusion level of 0 g, 1 g, 2 g and 3 g/kg diet. This was then mixed thoroughly and stored in a cool dry place. The experimental fish were fed at 3%, 5%, 7%, and 9% body weight at two weeks interval. Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and cumulative percentage survival and mortality rates was determined using the formulae below:

$$SGR = \frac{\text{Log final weight - Log initial weight}}{T_2 - T_1} \times 100$$

Log = Natural logarithm, T_2 = Time two and T_1 = Time one.

$$FCR = \frac{\text{Total feed consumed by fish (g)}}{\text{Total weight gain by fish (g)}}$$

$$\text{Percentage Mortality} = \frac{\text{Cumulative Mortality}}{\text{Total number stocked}} \times 100$$

$$\text{Percentage Survival} = \frac{\text{Cumulative Survival}}{\text{Total number stocked}} \times 100$$

2.3 Standard Plate Count (SPC) and Gut Sampling

Intestinal content of *Heteroclarias* was taken from 40 fish sampling and total plate count were enumerated using method described by Onwuka, [15]. Isolation of bacteria from *Heteroclarias* intestinal content after oral administration of probiotics for microbial count was carried out by taking one fish per replicate from each treatment, killed and gutted. Selective media (solid and broth media) was adopted according to method of Nebra and Blanch, [16]. Their gut content was obtained using a swab stick which was immediately transferred to 1 ml of 0.09% saline solution, thereafter homogenized in sterile distilled water. The homogenized sample was diluted serially up to (5×10^5) fold in sterile water and then inoculated on nutrient agar plates by pour plate method as described by Awan and Rahman, [17]. The inoculated plate was incubated at 37°C for 24 hours; the number of visible colonies was counted. The results were typically expressed as colony forming units (C.F.U.)/g. or /ML.

2.4 Haematological Examination

2.4.1 Blood sampling

5 ml of blood sample were collected from the experimental fish by severing them at the tail region and dripped blood samples into clean EDTA bottle. The blood samples were analyzed for Red Blood Cell (RBC), White Blood Cell (WBC) and Pack Cell Volume (PCV) according to the methods of (Kaplow, 1955) as described by Onwuka, [15]. Haematocrit value was determined by the standard micro haematocrit method, and expressed in percentage. Duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a micro haematocrit centrifuge at 12,000 rpm for 5 minutes and measured on a haematocrit reader.

2.5 Experimental Design and Statistical Analysis

Completely Randomized Design (CRD) was used for the experiment. The data obtained were subjected to one way analysis of variance (ANOVA) and all differences in mean values of parameters were determined at P = 0.05 level of significance. The coefficient regression equation was used to determine the length/weight relationships. Also Duncan Multiple range Test was used for mean separation.

3. RESULTS

The result in Table 1 shows that there was no significant difference ($p > 0.05$) of mean weight gain and feed conversion ratio among all treatments in this study. There was a significant difference ($p < 0.05$) in treatment I (0 g inclusion level probiotic drug) compared to others in terms of specific growth rate and percentage of survival. The result in Table 2 shows isolation and identification of bacterial organisms from *Heteroclarias* gut (stomach) fed probiotics inclusion level at 0, 1, 2 and 3 g/kg respectively.

It shows that standard plate count (SPC) differed significantly ($p<0.05$) among the treatments. The result of haematological parameters tested in the blood sample of experimental fish is indicated in Table 3. Result indicated shows that the red blood cell (RBC), white blood cell (WBC), pack cell volume (PCV) and haemoglobin (Hb) differed significantly ($p<0.05$) in between treatment I compared to other treatments. The result in Table 4 above shows that gram positive cocci was present in all the treatments as gram negative cocci were absent in all. However, gram positive rod was absent in treatment I as gram negative rod was present.

4. DISCUSSION

The least survival rate in the control could be attributed to the absence of beneficial microorganisms in the intestinal tract because the ability to control the micro flora in the intestinal tract with beneficial microorganisms was triggered by probiotics which increased the survival rate of *Heteroclarias* (Table 1). The combined effect of *Lactobacillus acidophilus* and *Bifidobacteria bifidum* used in this study as immune stimulant revealed to have enhanced the growth and resistance to disease of *Heteroclarias*, hence the higher percentage

Table 1. Growth response of *Heteroclarias* fingerlings Fed with probiotics at various inclusion levels for 56 days

Growth parameters	Treatment I	Treatment II	Treatment III	Treatment IV
Mean initial weight (MIW(g))	$1.88^{a}\pm0.75$	$1.86^{a}\pm0.05$	$1.79^{a}\pm0.38$	$1.87^{a}\pm0.04$
Mean final weight (MFW(g))	$15.84^{b}\pm0.27$	$20.67^{a}\pm0.82$	$19.92^{a}\pm0.24$	$20.10^{a}\pm0.96$
Mean weight gain (MWG(g))	$12.87^{b}\pm0.27$	$18.48^{b}\pm0.86$	$17.85^{b}\pm0.32$	$17.89^{b}\pm1.10$
Feed conversion ratio (FCR)	$0.65^{a}\pm0.02$	$0.64^{a}\pm0.06$	$0.57^{a}\pm0.04$	$0.62^{a}\pm0.41$
Specific growth rate (SGR(%/day))	$3.80^{b}\pm0.12$	$4.29^{a}\pm0.14$	$4.29^{a}\pm0.08$	$4.29^{a}\pm0.16$
% Survival rate	$63.33^{b}\pm2.8$	$85^{a}\pm5.00$	$86.66^{a}\pm2.88$	$85^{a}\pm5.00$

Legend: Treatment I=0 (g) probiotic per 1 kg diet; Treatment II= 1 (g) probiotic per 1 kg diet; Treatment III= 2 (g) probiotic per 1 kg diet and Treatment IV =3 (g) probiotic per 1 kg diet.
Mean data on the same row carrying different superscript differs significantly ($p<0.05$) from each other.

Table 2. Microbial count of gut sample of *Heteroclarias* fingerlings fed probiotics at various inclusion levels for 56 days

Microbial count	Treatment 1	Treatment II	Treatment III	Treatment IV
Colony forming unit (CFU)	$0.8^{a}x10^{5}\pm0.70$	$1.0^{b}x10^{5}\pm0.36$	$1.2^{c}x10^{5}\pm0.16$	$1.1^{c}x10^{5}\pm0.63$

Mean data on the same row carrying different superscripts differs significantly ($p<0.05$) from each other

Table 3. Haematological parameter of *Heteroclarias* fingerlings fed probiotic at various inclusion levels for 56 days

Blood Parameters	Treatment I	Treatment II	Treatment III	Treatment IV
Red blood cell (RBC($10^{6}mm^{-3}$)	$1.01^{a}\pm0.03$	$1.08^{b}\pm0.08$	$1.08^{b}\pm0.03$	$1.07^{b}\pm0.08$
White blood cell count (WBC($10^{3}mm^{-3}$)	$14.98^{a}\pm0.24$	$16.67^{b}\pm0.44$	$19.14^{c}\pm0.21$	$20.10^{c}\pm0.30$
Pack cell volume (% PCV)	$24.24^{a}\pm0.61$	$26.77^{b}\pm0.41$	$26.91^{b}\pm0.20$	$26.95^{b}\pm0.24$
Haemoglobin (Hb(g/100ml)	$8.96^{a}\pm0.39$	$10.11^{b}\pm0.20$	$11.80^{b}\pm0.15$	$11.66^{b}\pm0.33$

Mean data on the same row carrying different superscript differed significantly ($p<0.05$) from each other

Table 4. Gram staining for the culture of the gut content of *Heteroclarias* fed probiotic at various inclusion levels for 56 days

Bacteria Species	Treatment I	Treatment II	Treatment III	Treatment IV
Gram positive cocci	present	present	present	present
Gram positive rod	absent	present	present	present
Gram negative rod	present	absent	absent	absent
Gram negative cocci	absent	absent	absent	absent

survival in treatments II, III and IV. Similarly, [18] observed that a strain of *Lactoacillus rhamnosus* administered to rainbow trout enhanced survival rate and stimulates the respiratory boost activity after 2 weeks of feeding. The presence of gram positive rod shaped bacteria in *Heteroclarias* fed probiotics led to the establishment of a normal gut micro biota which add to the effective function of digestive system and serve as a barrier against invading pathogens as stated by Farzanfar, 2006 [19]. The high value of haematological parameters observed in the treatments fed probiotics on *Heteroclarias* showed that probiotics actively stimulate the proliferation of lymphocytes and immuneglobin production in fish as similar observation was made by [20].

5. CONCLUSION

From the research, it could be concluded that probiotics *(Lactobacillus acidophilus* and (*Bifidobacterium bifidum*) fed *Heteroclarias* at inclusion level of 1 g/kg diet was sufficient to stimulate immune and proliferate beneficial microorganisms in the gut.

6. RECOMMENDATION

Probiotics should be incorporated in commercial feeds at least 1 g/kg diet by fish farmers because of its ability to improve gut health and stimulating immune function.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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