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Effect of Probiotics-YUGE® on Survival and Growth of *Heterobranchus bidorsalis* Larvae Reared in Static-Renewal System

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ABSTRACT

Background and Objective: The poor survival and growth of *Heterobranchus bidorsalis* larvae recorded during rearing from post-yolk absorption to fingerlings in the hatchery have been a major problem brothering farmers in Northern Nigeria. This study was conducted to evaluate the efficacy of a commercial diet supplemented with commercial probiotics YUGE® in the rearing of *Heterobranchus bidorsalis* larvae to test if it will improve survival and growth in a static renewal system. **Materials and Methods:** Two weeks old larvae weaned on decapsulated artemia were reared on 0.0, 1.0, 2.0 and 3.0 g kg^{-1} levels of probiotics YUGE® supplemented commercial diet (Aqualis® fry powder) making up four dietary treatments allocated in triplicate in a completely randomized design. **Results:** Survival, growth and microbial constituent of larvae subjected to each treatment were monitored during four weeks of rearing. The result of the study showed that there was no significant difference ($p>0.05$) in the percent survival rate among the treatments. Larvae-fed probiotics YUGE® supplemented at 1.0 to 2.0 g kg^{-1} revealed higher growth and the highest *Bacillus* spp., recorded in gut microflora. **Conclusion:** It was concluded that the supplementation of the probiotics YUGE® in the diets of the larvae enhanced the percent occurrence of gut-beneficial *Bacillus* spp., at 1 to 2 g kg^{-1} of each which could have influenced the larvae's better performance in terms of survival and growth.

KEYWORDS

Growth, survival, *Heterobranchus bidorsalis*, larval, probiotics YUGE®, static-renewal, gut-microbial

INTRODUCTION

The sustainability of any aquaculture venture depends on several factors that include the availability of good quality seed and a foot greatly influenced by the availability of the right and less costly feed to attain the survival of larvae up to fingerlings. Starter feeds are increasing in cost by the day and continued trials of available supplements to ease feed utilization and enhance good feed conversion and survival become necessary. In Nigeria, catfish of the family Clariidae are widely distributed and the most cultured. However, the survival, growth and development during the nursery phase of larvae to juveniles are faced with a lot of mortality and poor growth. According to the research[1-3], before the larval period of development, larvae nutrition has been provided by the yolk sac and oil globule and after this, the organs are developed and function much as those in an adult fish. The transition from an endogenous to an exogenous food supply

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which marks the onset of the larval stage is one of the most critical phases of the life cycle and is the period when much of the mortality of hatchery-reared stock occurs. The requirement for a particular nutrient can be defined from a physiological point of view as the nutrient intake needed to fulfill a physiological role[4]. Fish require diets that are balanced in energy, protein, minerals and vitamins at all stages of development. The survival and growth rate of larvae during rearing is mostly influenced by the quality of feed and its acceptability, especially during intensive rearing[5], feeding constitutes a major factor since the fish obtain a good percentage of their nutritional requirements through the food they consume. The acceptability of feed depends on the feed type and particle size. Hence, an improvement in the artificial feed provided to ensure fry survival becomes very important. Nowadays, probiotics YUGE® have been widely used in aquaculture for the control of disease and also to increase feed efficiency and husbandry parameters[6]. The beneficial effects of these probiotics include higher growth and better feed efficiency, prevention of intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients. This study, therefore, tried to establish the influence of commercial probiotics YUGE® on the growth performance and intestinal microbial flora of *H. bidorsalis* larvae and which level of application in feed best enhances survival and growth performance in the hatchery.

MATERIALS AND METHODS

Study area: The experiment was carried out from June to July in the year 2017. This study was carried out at the Teaching and Research Fish Farm, Department of Fisheries and Aquaculture, Usmanu Danfodiyo University Sokoto. The site is on Latitude 13°07'78"N and Longitude of 05°12'25"E at 275 m above sea level. The site is located in the Sudan Savanah of Nigeria, with an agro-climate characterized by seven long dry months, occurring from October to April of every year, mean monthly maximum temperature of 31-40°C and mean monthly minimum temperature of 12-24°C and evapotranspiration of the order 1670 mm. The area is characterized by cool dry air during the harmattan from November to February and the hot season from March to May. Annual rainfall in the area ranged from 508 to 1016 mm/year[7]. The mean relative humidity is 14.9 and 40% in March and June, respectively.

Probiotics: The probiotic's composition and the manufacturer's detail were presented in Table 1.

Fish larvae: Larvae of the fish weaned from decapsulated artemia as starter feeds for two weeks in the teaching and research hatchery of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University Sokoto, were sampled and subjected to a feeding trial.

Experimental design, data collection and analysis: The experiment was carried out in 12 experimental units (70-liter capacity plastic bowls filled with clean water and fitted with a mechanical aerator) in a static renewal system (water exchange every 24 hrs). One hundred and fifty larvae (fry) were randomly sampled and stocked in each experimental unit. Diets (Aqualis® fry powder) supplemented with varying levels of YUGE®(0.0, 1.0, 2.0 and 3.0 g kg^{-1}) make up the dietary treatments (the manufacturer's recommended level of YUGE® for freshwater fish is 1-2 g kg^{-1} of feed) and allocated in triplicate making up to 4 experimental treatments arranged in a completely randomized design. The procedures applied in the data collection, data analysis and statistical analysis were carried out following the procedure by Abubakar and Ipinjolu[8].

Table 1: Name and manufacturer of probiotics used in this study

Probiotics	Composition	Manufacturer
YUGE®	*Bacillus subtilis*: 200 million CFU g^{-1} *Pediococcus acidilactici*: 100 million CFU g^{-1} Maifanite: 40% Starch: 35% Fermented *Aspergillus oryzae*: 20% Vitamin C: 5% Total viable bacteria: ≥300 million CFU g^{-1}	Shandong Baolai-Leelai Bio-Tech., Co., Ltd., China

Statistical analysis: Data collected on growth, survival, feed utilization and intestinal microflora were subjected to Analysis of Variance (ANOVA) and means were separated using New Duncan's Multiple Range Test (DMRT)[9]. Computer analysis was carried out using the SPSS V: 20.0 package for Windows.

RESULTS

The percent survival rate was not significantly ($p>0.05$) different among the four dietary treatments where those fed Treatment II had 60% and the least 51% was in Treatment IV, as presented in Table 2. The trend in the survival of the larvae in (Fig. 1) showed that larvae fed dietary treatment II (1 g kg^{-1}), maintained the highest mean survival (141, 136 and 91) throughout the experimental period (weeks 1 to 4) respectively, followed by those fed dietary treatment I (0 g kg^{-1}) except in the third week where that fed dietary treatment I, had the highest survival average of (110). The groups placed on dietary treatment III (2 g kg^{-1} feed) had low mortality with a mean survival rate of (89) in the 4th week (Fig. 1) of the experiment and had better survival than those on the treatment I and IV.

Table 2: Survival and growth indices of *Heterobranchus bidorsalis* larvae fed varying levels of probiotics YUGE® for 28 days

	Treatment/(g) probiotics/(kg) feed			
Parameter	TRT I (0 g kg^{-1})	TRT II (1 g kg^{-1})	TRT III (2 g kg^{-1})	TRT IV (3 g kg^{-1})
Initial fish number	450	450	450	450
Final fish number	256	274	267	232
Survival rate (%)	56.89±10.11	60.89±9.56	59.33±3.01	51.56±2.22
Initial body weight (mg)	67.78±0.44	67.56±0.97	68.22±0.22	67.77±0.44
Final body weight (mg)	423.49±40.87^{b}	526.99±33.65^{ab}	626.67±20.28^{a}	487.21±47.51^{b}
Weight gain (mg)	355.71±40.43^{b}	459.44±34.62^{ab}	558.44±20.26^{a}	419.43±47.18^{b}
Weight gain (%)	524.09±55.87^{b}	681.83±60.72^{ab}	818..57±29.72^{a}	618.22±66.70^{b}
Specific growth rate (%/day)	6.51±0.31^{b}	7.32±0.49^{ab}	7.92±0.20^{a}	7.01±0.12^{ab}
Initial body length (mm)	19.33±0.88	19.00±0.58	18.67±0.33	19.67±0.33
Final body length (mm)	44.00±2.52	47.33±2.96	48.00±0.58	47.00±0.99
Length increase (mm)	24.67±3.18	28.33±3.53	29.33±0.33	27.33±1.45
Length increase (%)	129.34±21.92	150.52±23.32	157.21±2.47	139.30±9.74
Condition factor (k)	0.52±0.11	0.51±0.08	0.57±0.04	0.48±0.08

Mean values in row with same letter are not significantly different ($p>0.05$), TRT: Treatment

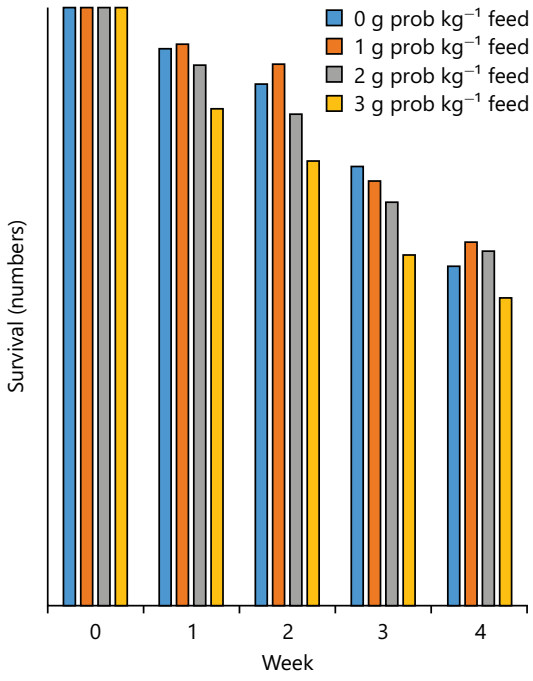

Fig. 1: Survival of *Heterobranchus bidorsalis* larvae reared on diets supplemented with different levels of probiotic YUGE® for 28 days

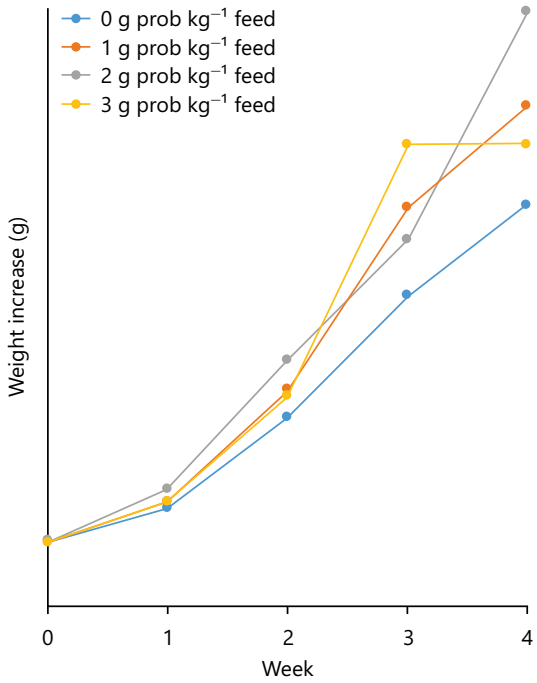

Fig. 2: Growth of *Heterobranchus bidorsalis* fed diets supplemented different levels of probiotics YUGE® for 28 days

The growth responses of the larvae are presented in Table 2. Larvae fed diet III (2 g kg^{-1}) had the highest weight gain (558.44±20.26) with no significant difference ($p>0.05$) from those fed diet II (1 g kg^{-1}) (459.44±34.62), but significantly ($p<0.05$) higher than the mean weight gains of 355.71±40.43 and 419.43±47.18 obtained for larvae fed diets I (control) and IV (3 g kg^{-1}), respectively. However, there was no significant ($p>0.05$) difference in the weight gained among larvae fed diets I, II and IV. The percent weight gained also followed the same trend. The specific growth rate (SGR (%)/day) indicates that larvae fed diet III (2 g kg^{-1}) had 7.92±0.20 which was significantly not different from those fed diets II (1 g kg^{-1}) and IV (3 g kg^{-1}). The control treatment recorded the least SGR but was not significantly different ($p>0.05$) from those fed diets II and IV. Results of the length increase and condition factor recorded were non-significantly ($p>0.05$) differences among the larvae subjected to the four dietary treatments.

The trend in the growth of the larvae on the different YUGE®-supplemented dietary treatments is as presented in Fig. 2. The graph showed that larvae fed dietary treatment IV (3 g kg^{-1}), maintained a competitive growth rate with those fed dietary treatment III and achieved the highest growth rate (42,426 mg) during the third week and declined in the fourth week of the experiment to (37,911 mg) when compared to other dietary treatments (II and III) that recorded higher weight increase 47,174 and 55,943 mg, respectively. However, larvae fed dietary treatments II and III maintained a competitive growth performance through the period of the trial but the latter showed better performance with increased weight gain than those on diet II (1 g kg^{-1}). Those fed the control dietary treatment I (0 g kg^{-1}) maintained the least weight gain throughout the testing period.

Results of the water quality parameters (temperatures, pH, dissolved oxygen and ammonia) during the period of the probiotic YUGE® experiment were presented in Table 3. The overall mean of the water temperature during the experimental period was 30.19±0.34°C with minimum and maximum values of 28.4 and 32°C respectively. The temperature varied from 27.3 to 30.9, with a mean of 29.07±0.33 in the morning, 29.5 to 32.1 with a mean of 31.06±0.25 in the afternoon and 28.4 to 33 with a mean of 30.44±0.45 in the evening. The mean pH during the experimental period was 7.75±0.09 and the value

Table 3: Mean water quality parameters measured during probiotics YUGE® experimental period

Parameter	Mean	Minimum	Maximum
Temperature (°C)			
Morning	29.07±0.33	27.3	30.90
Afternoon	31.06±0.25	29.5	32.10
Evening	30.44±0.45	28.4	33.00
Overall mean	30.19±0.34	28.4	32.00
PH	7.75±0.09	7.35	8.23
Dissolved oxygen (mg L^{-1})			
TRT I	4.62±0.32	4.62	5.39
TRT II	4.75±0.21	4.02	5.33
TRT III	4.61±0.15	4.22	5.00
TRT IV	4.71±0.13	4.25	5.00
Overall mean	4.67±0.20	4.03	4.18
Ammonia (mg L^{-1})			
TRT I	0.93±0.01	0.89	0.96
TRT II	0.94±0.02	0.87	0.98
TRT III	0.90±0.12	0.86	0.95
TRT IV	0.94±0.01	0.90	0.96
Overall mean	0.93±0.04	0.88	0.96

Mean values in row with same letter are not significantly different (p>0.05) and TRT: Treatment

Table 4: Mean number of cells (g^{-1} CFU) in the gut of *Heterobranchus bidorsalis* 28 days after feeding with diets supplemented with probiotics YUGE® at a different rate

	Bacterial count (CFU g^{-1})		
Treatment	Mean count	Minimum	Maximum
Initial	$4.3±0.6×10^5$	$3.0×10^5$	$5.0×10^5$
TRT I (Control)	$7.3±2.4×10^5$	$4.0×10^5$	$12.0×10^5$
TRT II (1 g YUGE® kg^{-1} of feed)	$5.3±1.7×10^5$	$2.0×10^5$	$8.0×10^5$
TRT III (2 g YUGE® kg^{-1} of feed)	$22.3±1.7×10^5$	$5.0×10^5$	$57.0×10^5$
TRT IV (3 g YUGE® kg^{-1} of feed)	$7.3±2.0×10^5$	$4.0×10^5$	$11.0×10^5$

Mean values in row with same letter are not significantly different (p>0.05) and TRT: Treatment

Table 5: Frequency of occurrence of bacterial isolates

Bacterial isolate	Frequency of occurrence	Occurrence (%)	Bacterial type (%)	
Proteus vulgaris	1	6		
Salmonella typhi	1	6	*Bacillus* spp.	42.0
Citrobacter fruendii	1	6		
Bacillus firmus	2	12		
Bacillus subtilis	3	18		
Salmonella paratyphia	1	6	Other bacterial	58.0
Listeria monocytogenes	3	18		
Micrococcus kristinae	1	6		
Kurthia spp.	1	6		
Bacillus lentus	2	12		
Morganella morganii	2	12		
Total	18	100		100

ranged from 7.35 to 8.23. The mean and the range of dissolved oxygen and ammonia concentrations were 4.67±0.20 (4.03-4.18) and 0.093±0.04 (0.088-0.096), respectively. There were no significant differences among the means of the water quality parameters among the four treatments.

The mean bacterial counts and frequency of occurrence of bacterial species isolated from the larvae gut fed different dietary treatments containing probiotics YUGE® are presented in Table 4 and 5. The initial bacterial counts from the larvae before subjecting them to probiotics YUGE® was observed to be lower than $4.3±0.6×10^5$ and this was lower than the load counted after feeding them probiotics YUGE®. The

highest mean count obtained after the trial was in treatment II (1 g YUGE® kg^{-1} of feed) ($22.3\pm1.7\times10^5$) followed by those in treatments I (Control) and IV (3 g YUGE® kg^{-1} of feed) with $7.3\pm2.4\times10^5$ and $7.3.0\pm2.0\times10^5$, respectively, while the least was in treatment II (1 g YUGE® kg^{-1} of feed) $5.3\pm1.7\times10^5$.

The total number of bacteria counts in larvae subjected to probiotic YUGE® supplemented diets was eighteen. *Bacillus subtilis* and *Listeria monocytogenes* accounted for the highest frequency of 18% followed by the occurrence of *Bacillus firmus, Bacillus lentus* and *Morganella morganii* with 12% each. The least occurring were *Proteus vulgaris*, *Salmonella typhi*, *Citrobacter fruendii*, *Salmonella paratyphi A*, *Micrococcus kristinae* and *Kurthia* spp., each with 6% occurrence. The *Bacillus* spp., accounted for 42% while other bacterial groups scored 58% of the total occurrence.

DISCUSSION

The growth and survival of *H. bidorsalis larvae* on probiotics YUGE®, showed that there was no significant difference ($p>0.05$) in percent survival rate among the treatments. The survival trend observed in this study was observed to be similar to the trend observed by Pooramini *et al.*[10]. The best weight-gain obtained in TRT II in this study could be influenced by the microbial floral change, where bacillus species were recorded to be highest in the gut of larvae fed 2 g kg^{-1} YUGE® and evidence of dominant colonization. This was similar to the findings of researchers[11-13] where administration of probiotics significantly changed the proportion of *Bacillus* bacteria in the gut flora of freshwater fish. Bairagi *et al.*[14], showed that two specific strains of fish intestinal bacteria, *Bacillus subtilis* and *B. circulans*, having extracellular cellulolytic and amylolytic activities, were used to inoculate duckweed (*Lemna polyrhiza*) leaf meal and had the best effects on the growth and feeding efficiency of rohu fingerlings. The early intestinal colonization of the gut of a fish by the probiotic may have some effect on development, by accelerating the maturation of the digestive system and stimulating metabolism and growth[12,15]. The presence of more *Bacillus* spp., which is known to produce digestive proteases and other enzymes that enable it to contribute to the natural digestion activity of the cultured species and it can be a source of micro and macro-elements as feed[16-18] up to 42% (Table 5), could have enhanced better growth recorded in the larvae fed probiotics YUGE® supplemented diet. The sudden drop in the growth (Fig. 2) of larvae subjected to dietary treatment IV (3 g kg^{-1}) in the fourth week could be a sign of a limit in the level of exposure of the larvae to the high level of probiotics YUGE® supplement. This buttresses the finding of Bagheri *et al.*[19], who observed that the presence of the largest probiotics YUGE® cells in diets and host intestine necessarily does not result in the ppdimproved growth and survival of *Onchorrhynchus mykiss* fry. This further confirms the necessity for proper supplementation of the correct probiotics YUGE® quantity to function effectively in the diets of fish larvae.

Implications: To ensure effective supplementation of basal diet with probiotics YUGE®, it is recommended that *H. bidosalis* fry be fed at 1 to 2 g kg^{-1}, for better utilization of basal feed and stimulation of productive performance. The results of this investigation will be helpful in formulation of practical diets for culture of fry.

CONCLUSION

The findings from the study showed that different levels of probiotics YUGE® could cause different effects on growth parameters in *Heterobranchus bidorsalis* larvae. Better growth was obtained in larvae fed at 1 and 2 g kg^{-1} of basal feed. It can be concluded that the supplementation of the probiotics YUGE® in the diets of the larvae enhanced the percent occurrence of gut beneficial *Bacillus* spp., at 2 g kg^{-1} which could have influenced the larvae performance in terms of survival and growth when reared in a static renewal water exchange system.

SIGNIFICANCE STATEMENT

This study discovered that probiotics YUGE® can be beneficial for enhancing the survival and growth of *Heterobranchus bidorsalis* post yolk absorption larvae in a static renewal water exchange system. This study will help the researchers to uncover the critical areas of probiotics YUGE® that many researchers were yet able to explore on its efficacy on *Heterobranchus bidorsalis*.

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