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Growth Response and Nutrient Utilization of *Heterobranchus longifilis* Fingerlings Fed Diets Fortified with *Lactobacillus Paracasei*

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Abstract

A 12-week feeding trial was conducted in 12 plastic tanks (50cm x 34cm x 27cm) to assess the performance of *Heterobranchus longifilis* fingerlings fed diets containing *Lactobacillus paracasei* at different inclusion levels. Four diets were formulated at 40% crude protein content containing: no *L. paracasei* (control), *L. paracasei* at 2.0 x 10^8 cfu/ml (LPA1), *L. paracasei* at 4.0 x 10^8 cfu/ml (LPA2), *L. paracasei* at 6.0 x 10^8 cfu/ml (LPA3). Each treatment was done in triplicate containing 20 fish (mean weight of 3.05±0.02g) each. Fish were fed to satiation. *Heterobranchus longifilis* fed LPA1 had significantly higher mean weight gain, specific growth rate, Protein efficiency ratio, nitrogen metabolism, and the lowest feed conversion ratio of 29.36±0.51, 1.22±0.01, 38.47±0.42, 15.39±0.17, 1.91±0.01, 817.41±11.56 and 1.31±0.01, respectively. The result from this study indicates that dietary *L. paracasei* at inclusion level of 2.0 x 10^8 cfu/ml could enhance the growth and nutrient utilization of *Heterobranchus longifilis.*

Keywords: *Heterobranchus longifilis*, *Lactobacillus paracasei*, inclusion level, growth, nutrient utilization.

Introduction

Aquaculture which is the cultivation of aquatic organisms in natural water bodies, semi-controlled and controlled environment is essential to satisfy the increase in fish demand. Although it has been reported that aquaculture accounted for 52% of fish for human consumption (FAO, 2020), the sector is still faced with a major challenge of the availability and high cost of quality feed (Aba, 2020). Fish feed represents about 50% - 60% of total production (Omitoyin *et al*., 2006). It is, therefore, needful to produce feed that would meet the nutritional requirement of the fish, improve the digestibility of nutrients and achieve profitable growth within the shortest period without any negative effect on the fish.

Catfishes of the family Claridae (*Clarias* and *Heterobranchus*) comprise the most commonly cultivated fishes in Nigeria. They are economically important food fish in Nigeria and other parts of the world and command a very good commercial value in the market (Ndome *et al*., 2011). *Heterobranchus longifilis* like other catfish in this family can grow on a wide range of natural and low-cost artificial feed and can withstand low oxygen and pH levels. Its other attributes include resistance to disease, high yield potentials, high fecundity, high palatability, fast growth rate in various culture systems, ability to withstand unfavourable environmental conditions, and simple techniques in the propagation of their fingerlings (Oworinde and Ndimele, 2011).

The need to improve fish growth has led to the use of various growth-promoting compounds such as probiotics, prebiotics, synbiotics, phytobiotics, and other functional dietary supplements. Supplementing diets with these feed additives promotes growth performance, feed utilization, and net financial return (Mohsen *et al*., 2016).

Recently, there is more focus on research centred on the use of probiotics in aquaculture as a result of its beneficial impacts. Its use as a feed supplement has attracted considerable attention from feed manufacturers as a means of improving livestock performance (Renuka *et al*., 2014). Probiotic is defined as live microorganisms that when administered in adequate amounts, confer benefit to the host's health by improving the overall balance of the ambient microbial community, ensuring improved use of feed, and enhancing the host's response to disease (Abdelhamid *et al*., 2009).

Probiotics have numerous beneficial effects on fish culture such as improving the activity of gastrointestinal microbiota, enhancing immune status, disease resistance, survival, feed utilization, and growth performance, and also offer a safer natural way of raising fish stock (Bidhan *et al*., 2014). However, the right probiotic strain to use will differ according to the species of fish being cultured and the water quality of the culture medium. Probiotics may show host-specific and strain-specific differences in their activities (Nayak, 2010). It is therefore necessary to choose the right strains of probiotics that will produce beneficial effects for a particular fish species. Commonly used probiotics in aquaculture include Lactic acid bacteria (*Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus* bacteria, and *Enterococcus*), the genus Vibro, *Bacillus* and *Pseudomonas*, etc.

More information is needed on the effect of specific probiotics on cultured fish species like *Heterobranchus spp, Oreochromis niloticus* (Nwanna *et al*., 2013), and other cultured fish species in Nigeria. There is little information on the use *of Lactobacillus paracasei* and its optimum level of inclusion in the feed of *Heterobranchus longifilis* fingerlings. This study was conducted to assess the performance of *Heterobranchus longifilis* fingerlings fed diets containing *Lactobacillus paracasei* at different levels of inclusion.

Materials and Methods

Diet preparation

Fish feed ingredients were procured from Adom Plaza feed store, at Ajibode junction, Ibadan. *Lactobacillus paracasei* HBUAS 53050 was isolated (from 'Wara' fermented cow milk) and biochemically characterized at the Department of Microbiology, University of Ibadan, Ibadan, using standard procedures (Iranmanesh *et al*., 2014; Todorov *et al*., 2011; Mourad and Eddie, 2006; Simova *et al*., 2009). *Lactobacillus paracasei* HBUAS 53050 was molecularly characterized using standard procedures (Saraniya and Jeevaratnam, 2012; Agaliya and Jeevaratnam, 2013) at The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

A basal diet of 40% crude protein was prepared using Pearson Square Method (Table 1). *L. paracasei* was added to experimental feed according to each treatment (2.0×10^8 CFU/mL, 4.0×10^8 CFU/mL, 6.0×10^8 CFU/mL). Feed ingredients were thoroughly ground, mixed, and pelleted using water and a binding agent (starch). Feed pellets were sun-dried, packed in a plastic container with a cover, and stored in a cool dry place. The feeds were reproduced after 28 days to avoid nutrient depletion.

Viability Test

The viability test of *Lactobacillus paracasei* in the formulated diets was carried out to ascertain how viable they are within the feed. It was done thrice during the feeding trial at the Department of Microbiology, University of Ibadan, Ibadan.

Table 1: Gross Composition of Experimental diets (kg)

Feed Ingredients	Treatments			
	Control	LPA1	LPA2	LPA3
Fish meal	12.76	12.76	12.76	12.76
Groundnut cake	25.52	25.52	25.52	25.52
Soybean meal	38.28	38.28	38.28	38.28
Yellow maize	6.65	6.65	6.65	6.65
Guinea corn	13.3	13.3	13.3	13.3
Palm oil	1	1	1	1
Mineral premix	1	1	1	1
Vitamin premix	0.5	0.5	0.5	0.5
Starch (binder)	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100
LPA (X 10^8) CFU/mL	0	2.0	4.0	6.0

Note: LPA = *Lactobacillus paracasei*

KEYS

Control - Basal diet of 40% crude protein level
LPA1 - Basal diet with additional *Lactobacillus paracasei* from Wara at 2.0 x 10^8 CFU/mL
LPA2- Basal diet with additional *Lactobacillus paracasei* from Wara at 4.0 x 10^8 CFU/mL
LPA3 - Basal diet with additional *Lactobacillus paracasei* from Wara at 6.0 x 10^8 CFU/mL

Experimental Design and Procedure

This study was carried out in the research laboratory of the Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria. Two hundred and forty (240) *Heterobranchus longifilis* fingerlings with an average weight of 3.05 ±0.02g were purchased from a reputable fish farm in Sango Otta, Ogun State, Nigeria and transported in a well-oxygenated bag half-filled with water to the Department of Aquaculture and Fisheries Management Research Laboratory. Fish were acclimatized for 2 weeks and fed with commercial feed, after which the feeding trial commenced.

Fish were distributed equally into 12 plastic aquaria with a dimension of 50cm x 34cm x 27cm and 40 litres capacity of water. The water level in each aquarium was maintained at 30 litres throughout the 12 weeks of the experiment and replaced every 2 days to maintain the relatively uniform physio-chemical parameters and prevent fouling from feed residues. The source of water used for the experiment was a borehole at the University of Ibadan. Each aquarium was well aerated using air stones and aerator pumps (Cosmos aquarium air pump, double type 3500 50 Hz, 2.5 – 3W) as described by Lawson (1995).

Fish were fed twice daily at 8.00 and 17.00 hours. Measurement of fish weight was carried out fortnightly using a sensitive scale (Model: SF-400C) of 0.01g – 500g. The dissolved oxygen of the experimental tanks was monitored using a dissolved oxygen meter (Jenway 3015DO meter, 0.01 accuracy, Genway, Staffordshire, UK). The water temperature of the experimental tanks was monitored using a mercury-in-glass thermometer (producer: Paragon Scientific Ltd., Birkenhead, Wirral, UK) while the pH meter (Jenway 3015 pH meter, 0.01 accuracy, Genway, Staffordshire, UK) was used in measuring the pH of water in the experimental tanks. The dissolved oxygen, temperature, and

pH of the culture medium were measured daily (Table 2).

Biological Evaluation

Growth parameters were monitored during the experimental period according to Hassan and Ngaski, 2007.

Mean weight gain (MWG)

$MWG = (W_2-W_1)g$, Where W_1 = initial mean weight (g), W_2 = final mean weight (g)

Specific growth rate (SGR)

$$SGR\ (g/day) = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1} \times \frac{100}{1}$$

W_2 = Final weight of fish, W_1 = Initial weight of fish, T_2 = Final time, T_1 = Initial time

e = Natural logarithm, T_1 and T_2 = experimental period in days

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry weight of feed consumed(g)}}{\text{Fish weight gain (g)}}$$

Protein efficiency ratio (PER)

$$PER = \frac{\text{Mean weight gain}}{\text{Protein intake}}$$

Where Protein intake (PI) = Feed intake x Protein in the diet (40% CP)

Survival rate (SR, %)

$$SR\ \% = \frac{\text{Initial number of } \textit{H. longifilis} \text{ stocked} - \text{mortality}}{\text{Initial number of } \textit{H. longifilis} \text{ stocked}} \times 100$$

Nitrogen metabolism (NM)

$$NM = \frac{(0.549)(a+b)}{h}$$

Where a = initial mean of fish, b = final mean weight of fish, h = experimental period in days, 0.549 = Constant.

Proximate Analysis

The experimental diets were subjected to proximate analysis on dry matter bases. Moisture %, crude protein %, ether extract %, crude fibre %, ash %, and nitrogen-free extracts (NFE) % contents were analyzed according to the official methods of analysis described by the Association of Official Analytical Chemist (AOAC, 2005).

Statistical Analysis

Data were subjected to a one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS, 2006 version 20.0). Duncan's multiple range test (DMRT, 1955) was used to compare differences among individual treatment means at ($P>0.05$). Regression analysis was used to determine the optimum concentration of *L. paracasei* for the growth of *H. longifilis*. The results were presented as means ± standard deviation of three replicates.

Results

The water quality of experimental tanks of fish-fed *Lactobacillus paracasei* at different inclusion levels is presented in Table 2. There were no significant differences ($p<0.05$) among the treatment groups. All the recorded values of dissolved oxygen, temperature, and pH were within the recommended range of water quality for the culture of catfish.

The proximate composition of experimental feed is presented in Table 3. The result showed that feed fortified with *L. paracasei* had significantly higher moisture content and higher nitrogen-free extract than the control feed. The significantly higher crude protein (44.29±0.13%) was recorded in LPA 3

and lowest in the control diet (43.81±0.05%), however, there was no significant difference in the crude protein of LPA2 and the control diet. The crude fibre was highest in LPA3 (6.91±0.01%), control (6.81±0.03%) and lowest in LPA1 (6.51±0.01%). There were no significant differences ($p>0.05$) in the ash content of LPA2 (6.79±0.04%), LPA3 (6.83±0.03%), and control (6.82±0.03%), while the LPA1 diet (6.71±0.01%) had the lowest ash content. Nitrogen-free extract content was significantly different across the diets, LPA2 had the highest (38.38±0.06%) while the control had the least (37.45±0.12%). Ether extract content was significantly higher in the control diet (5.00±0.02%) while the LPA3 diet had the least (4.32±0.02%).

Table 4 shows the proximate composition of *H. longifilis* fed diets fortified with *L. paracasei* for 12 weeks. The result showed that moisture, crude protein, ether extract, and ash contents were significantly different across the groups. *H. longifilis* treated with LPA3 diet had the highest moisture content (78.26±0.01%) while control fish had the least (75.19±0.01%). The highest crude protein was recorded in the fish fed LPA2 diet (18.65±0.02%) while the least (16.52±0.02%) was recorded in fish fed LPA1 diet. The ether extract was higher in fish fed with the control diet (3.83±0.01%) and least (2.63±0.01%) in fish fed with the LPA1 diet. Fish fed the control diet had the highest ash content (2.84±0.01%) while those fed with LPA3 diet had the least ash content (2.30±0.01%). There were no significant differences in the NFE of *H. longifilis* fed with diets LPA2, LPA3, and control. Fish fed LPA1 diet (0.51±0.02%) had significantly higher NFE.

Table 2. Water Quality Parameter of Culture Media of *Heterobranchus longifilis* Fed Diets Fortified with *Lactobacillus paracasei* for 84 Days

Parameter	Control	LPA1	LPA2	LPA3
Temperature	27.37±0.13	27.36± 0.12	27.37± 0.15	27.37±0.10
Dissolved Oxygen	5.37± 0.10	5.36±0.10	5.36±0.10	5.37±0.11
pH	7.50±0.03	7.49±0.03	7.49±0.03	7.49±0.02

Table 3. Proximate Analysis of Feed Fortified with *Lactobacillus paracasei* at Different Levels of Inclusion (%)

	Control	LPA1	LPA2	LPA3
Moisture	$8.93±0.04^{b}$	$9.90±0.04^{a}$	$9.87±0.03^{a}$	$9.97±0.03^{a}$
Crude Protein	$43.81±0.05^{b}$	$44.03±0.17^{ab}$	$43.90±0.6^{b}$	$44.29±0.13^{a}$
Crude Fat (EE)	$5.00±0.02^{a}$	$4.45±0.02^{b}$	$4.49±0.02^{b}$	$4.32±0.02^{c}$
Crude Fibre	$6.81±0.03^{a}$	$6.51±0.01^{b}$	$6.39±0.01^{c}$	$6.91±0.01^{a}$
Ash	$6.82±0.03^{a}$	$6.71±0.01^{b}$	$6.79±0.04^{a}$	$6.83±0.03^{a}$
NFE	$37.45±0.12^{d}$	$38.24±0.19^{b}$	$38.38±0.06^{a}$	$37.60±0.12^{c}$

Means with different superscripts within the same row were significantly different ($P<0.05$), while the absence of letters means not significantly different ($P>0.05$). NFE = Nitrogen free extract

Table 4. Proximate Analysis of *Heterobranchus longifilis* fed *Lactobacillus paracasei* Fortified Diet for 84 Days (%)

	Control	LPA1	LPA2	LPA3
Moisture	75.19±0.01^{d}	77.96±0.02^{b}	76.17±0.02^{c}	78.26±0.01^{a}
Crude Protein	18.14±0.01^{b}	16.52±0.02^{c}	18.65±0.02^{a}	16.59±0.03^{c}
Crude Fat (EE)	3.83±0.01^{a}	2.63±0.01^{d}	2.78±0.01^{c}	2.85±0.01^{b}
Ash	2.84±0.01^{a}	2.39±0.02^{c}	2.41±0.01^{bc}	2.30±0.01^{d}
NFE	0.00±0.00^{b}	0.51±0.02^{a}	0.00±0.00^{b}	0.00±0.00^{b}

Means with different superscripts within the same row were significantly different (P<0.05), while the absence of letters means not significantly different (P>0.05). NFE = Nitrogen free extract

As observed in Table 5 mean weight gain (MWG), specific growth rate (SGR), feed intake (FI), protein intake (PI), protein efficiency ratio (PER) and nitrogen metabolism (NM) were significantly higher in fish treated with *L. paracasei*. The highest MWG (29.36±0.51 g), SGR (1.22±0.01), FI (38.47±0.42), PI (15.39±0.17), PER (1.91±0.01), and NM (817.41±11.56) were recorded in *H. longifilis* fed LPA1 diet and the least were obtained in fish fed the control diet. FCR was significantly higher in the fish fed with control diet (1.59±0.03) and least in the fish fed LPA1 diet (1.31±0.01). The relationship between the SGR of *H. longifilis* and the inclusion level of *L. paracasei* was expressed by the second-order quadratic regression (R^2 = 0.99). From the graph, optimum inclusion level 200076125.0000cfu ($2.00x10^8$) resulted into the highest SGR of 1.22%/fish/day. There were no significant differences in the survival rate of fish in all the groups.

Table 5. Growth Parameters of *Heterobranchus longifilis* Fed Diets Fortified with *Lactobacillus paracasei* for 84 Days

	Control	LPA1	LPA2	LPA3
Initial weight (g)	3.05±0.03	3.04±0.03	3.03±0.03	3.06±0.04
Final weight (g)	23.09±0.25^{c}	32.41±0.50^{a}	27.77±0.27^{b}	27.60±0.41^{b}
Mean weight gain(g)	20.05±0.28^{c}	29.36±0.51^{a}	24.74±0.29^{b}	24.53±0.40^{b}
Specific growth rate	1.05±0.01^{c}	1.22±0.01^{a}	1.15±0.01^{b}	1.14±0.01^{b}
Feed intake	31.86±0.96^{d}	38.47±0.42^{a}	34.18±0.94^{c}	34.47±0.79^{bc}
FCR	1.59±0.03^{a}	1.31±0.01^{d}	1.38±0.02^{c}	1.40±0.02^{bc}
Protein intake	12.74±0.39^{d}	15.39±0.17^{a}	13.67±0.38^{c}	13.79±0.32^{bc}
PER	1.57±0.03^{d}	1.91±0.01^{a}	1.81±0.03^{b}	1.78±0.02^{bc}
Nitrogen metabolism	602.74±6.47^{c}	817.41±11.56^{a}	710.34±5.54^{b}	706.96±9.67^{b}
Survival rate (%)	90.00±4.47	91.67±2.89	90.00±0.00	91.67±7.64

Means with different superscripts within the same row were significantly different (P<0.05), while the absence of letters means not significantly different (P>0.05). FRC = Feed conversion ratio, PER = Protein efficiency ratio

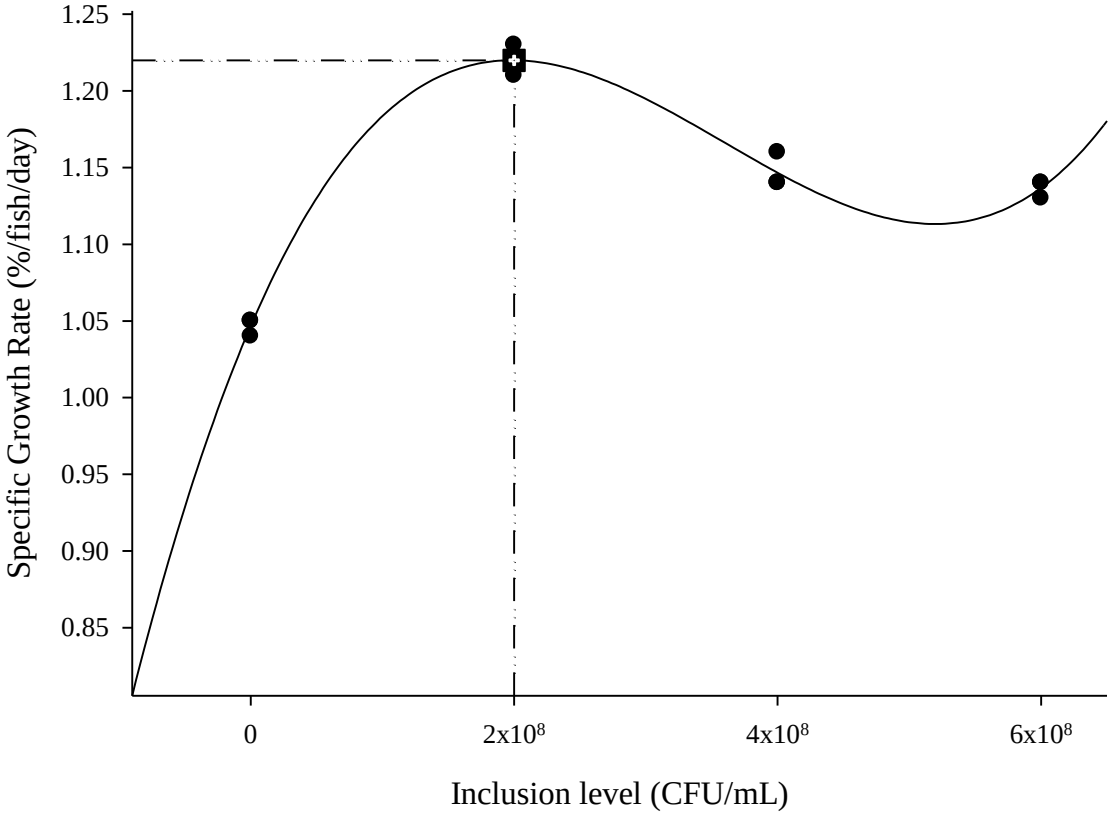

Figure 1. The specific growth rate of *H. longifilis* fed *L. paracasei* fortified diet at different inclusion levels

Discussion

In the present study, the weekly mean water quality parameters: Temperature (^{0}C), Dissolved oxygen (DO), and pH were within those recommended by Bhatnagar and Devi (2013) for pond water fisheries, which are Temperature; 15-35^{o}C, Dissolved oxygen; 3-5 mg/L, pH; 7-9.5.

The inclusion of *L. paracasei* at different levels in the diet of *H. longifilis* produced variations in the proximate analysis and nutrient composition of the diets. These variations could have led to different levels of nutrient utilization by the experimental fish. However, the crude protein levels in the diets fall within the recommended range for catfish (38% - 42%) according to Olaifa *et al*., 2013).

The inclusion of *L. paracasei* in the diet of *H. longifilis* significantly increased the MWG, SGR, FI, PI, PER, and NM of the groups when compared with the control stock. Also, fish fed diets fortified with *L. paracasei* had lower FCR which indicates more efficient utilization of the diets by the fingerlings. Higher SGR observed in fish fed diet fortified with *L. paracasei* signifies the well-being robustness of fish (Ibrahim *et al*., 2010). An increase in feed intake could be a result of stimulated appetite and the acceptability of the feed to *H. longifilis*. Abd El-rhman *et al*., (2009) noted that probiotics can stimulate appetite and improve

nutrition by producing vitamins and detoxifying toxic compounds in the diet, and by breaking down indigestible components. Increased protein efficiency ratio in *L. paracasei* fortified diets when compared to the control group indicates better utilization of protein for growth in fingerlings fed the diet.

Better performance in growth and nutrient utilization of fish fed diets supplemented with *L. paracasei* compared with fish fed control diet is in line with the results obtained in other studies (Mohapatra *et al*., 2012, Nwanna *et al*., 2011, Ayoola *et al*., 2013, Nwanna and Tope-Jegede, 2017) using different types of probiotics individually or in combination. Seenivasan *et al*., (2013) and Adesina *et al*., (2018) reported higher growth rates in freshwater prawn fed *L. sporogenes* and *L. acidophilus* respectively. Sha *et al*., (2016) also reported that the use of Lactic acid bacteria (LAB) improved the growth performance of shrimp (*Litopenaeus vannamei*).

The survival rates of fish across the group were not significantly different. It was generally high in all treatment groups.

However, fish fed LPA1 diet (2.0 x 10^8 CFU/mL inclusion) had significantly highest MWG, SGR, FI, PI, PER, and NM when compared to fish fed LPA2 diet (4.0 x 10^8 CFU/mL inclusion) and LPA3 diet (6.0 x 10^8 CFU/mL inclusion) in the present study. This indicates that there is an optimum level of probiotic inclusion in the diets, that is, there should be a limit to probiotic inclusion, beyond which it should be considered inadequate or excessive leading to undesirable results. The optimum inclusion level may likely vary with fish species and strain of probiotics being used. Nayak, 2010 reported that probiotics may show host specific and strain specific differences in their activities.

Conclusion

This study reveals that feed fortified with *Lactobacillus paracasei* enhanced the growth and nutrient utilization of *Heterobranchus longifilis* fingerlings. Inclusion level of 2.0 x 10^8 CFU/mL yielded the best result when compared with inclusion levels at 4.0 x 10^8 CFU/mL and 6.0 x 10^8 CFU/mL respectively.

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