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Probiotic Supplementation Improved the Growth and Health Status of Nile Tilapia

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Abstract

Aquaculture industry is the fastest-growing food production sector supporting almost 50% of all aquatic foods for human consumption. This study was aimed probiotic supplementation to improve the growth and health status of Nile tilapia. Fingerlings with mean initial body weight of 11.43 ± 1.27 g and mean initial body length of 6.2 ± 0.84 cm were stocked in 12 happas. They fed four times a day diet contains 30% of crude protein at 5% of their body weight and reared for two months. The four treatments were triplicated as a control diet, and the three experimental diets fortified with *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and their combinations at a dosage of (1.5×10^8 CFU/ml) fed for two rearing months. Growth performance and gut pathogenic microbes of fish were investigated. Results indicated that the highest growth performance was achieved in treatments of *Lactobacillus fermentum* with body weight gain of 33.43 ± 1.65 g, daily growth rate of 0.51 ± 0.05 g, feed conversion ratio 1.89 ± 0.10, protein efficiency ratio, 1.78 ± 0.09 whereas, the lowest growth performance was achieved in treatments fed with control diet 19.83 ± 2.23, 0.33 ± 0.04, 3.52 ± 0.39, and 0.97 ± 0.11 respectively. Pathogens like *Salmonella*, *Shigella*, *E. coli* and *Proteius* were detected when sampled fish were gut dissected for investigation of Nile tilapia (*O. niloticus*) of treated with fish group treated with control diet and these pathogens were not detected in fish group treated with experimental diet post-treatment with probiotics. Finally, study confirmed that supplementation of probiotics in diets enhanced the growth performance, nutrient utilization, feed conversion ratio and improved health status than fish fed on the control diet ($P \leq 0.05$) of Nile tilapia (*O. niloticus*) reared under pond culture condition.

Keywords: Aquaculture; Gut micro flora; Health; Nile tilapia; Probiotics

Introduction

Aquaculture industry is the fastest-growing food production sector supporting almost 50% of all aquatic foods for human consumption. Ethiopia is one of the developing countries where food security is not ensured yet and there is a need to expand aquaculture. Ethiopian universities, research organizations and fish farmers from different regions are now practicing aquaculture. Infectious pathogens impose formidable limitations on the growth and sustainability of the aquaculture industry, interfering economically and reducing the quality of life for common people. To address these problems, several antibiotics have been administered in the diets of cultured aquatic species.

The generic use of antibiotics for disease preventative purposes leads to selection favoring antibiotic-resistant pathogens, with risks of transmission to terrestrial animals; these troublesome organisms can eventually enter the human food chain. This potentially poses a health threat to consumers of seafood's cultured with antibiotics. For these reasons, alternative approaches are urgently needed to address the threat of pathogens in aquaculture. Probiotics can be environmentally and consumer-friendly alternatives to confront this critical situation. These can not only enhance growth and improve feed utilization, but also enhance immune responses; these favorable results are often accompanied by changes in intestinal morphology and microbial status of cultured species. Several studies revealed that supplementation of the associated feed additives greatly modulate the microbial scenario of fish. Probiotics can be incorporated into fish diets to stimulate growth and to protect from various pathogenic diseases, and many are increasingly viewed as potential substitutes for antimicrobial agents.

The Nile tilapia's (*O. niloticus*) has grown in phenomenally in popularity and has become the second most abundantly farmed fish, behind carps ast worldwide. The rearing of this species is produced in large quantities at worldwide, due to its modest requirement for artificial feeds, its tolerance of water quality and environmental variation, short crop cycle, absence of intramuscular bone, and high resistance to most pathogens [1]. Moreover, in Ethiopia majority of fish farmers rear Nile tilapia's (*O. niloticus*) due to its fast growth potential and high preference for market demand by the society. Though a number of studies demonstrated that probiotic yeast (*Saccharomyces cerevisiae*) improved the growth performance, stress tolerance, immunity and disease resistance in Nile tilapia (*O. niloticus*). None of those has specifically addressed the role of *Saccharomyces cerevisiae* and *Lactobacillus fermentum* at individual supplementation and its combined effect in the morphological modifications of Nile tilapia's (*O. niloticus*) Gastrointestinal Tracts (GIT) and its health status. Therefore, the present study was aimed to investigate probiotic supplementation improved the growth and health status of Nile tilapia's (*O. niloticus*).

Materials and Methods

Description of the study area

This study was conducted at Centre for Aquaculture Research and Education (CARE), College of Natural and Computational Sciences, Hawassa University. CARE is located at 275 km south of Addis Ababa, the capital city of Ethiopia. The CARE is situated at 7°3'7"N latitude and 38°3'17"E longitude with an altitude of 1714 m.a.s.l.

Study design

Nile tilapia (*O. niloticus*) fingerlings used in this experiment were collected from CARE. Three hundred (300) Nile tilapia (*O. niloticus*) fingerlings were collected from a culture pond. They were stocked in 12 happas each contains 25 fingerlings in four treatment groups. Beside their treatment group 300 fingerlings were stocked in each happas. The fingerlings were stocked with a mean initial body weight of 11.43 ± 1.27 g and mean body length of 6.2 ± 0.84 cm. Treatment-1 from 1-3 happas contains only control diet, treatment-2 from 4-6 diet fortified with *Saccharomyces cerevisiae* at a dosage of (1.5×10^8 CFU/ml), treatment-3 from 7-9 was fortified with *Lactobacillus fermentum* at a dosage of (1.5×10^8 CFU/ml) and treatment-4 from 9-12 diet fortified with *Saccharomyces cerevisiae* and *Lactobacillus fermentum* combined at a dosage of (1.5×10^8 CFU/ml). Fingerlings were acclimatized in the happas for 15 days before conducting the experiment. They were fed with control diet containing 30% Crude Protein (CP) at 5% of their body weight. During this period, fishes were adapted on feeding of control diet (without any additives). Half volume of pond water was changed once a week to maintain good water quality by removing wastes. Improving water quality, avoids the accumulation of organic, nitrogen, ammonia, and nitrite waste are constant concerns in aquaculture. Changing water once a week was not much enough to remove wastes accumulated in the aquaculture. Moreover, during investigation when the fingerlings removed from happas for morphometric measurement investigator always cleaned happas where fingerlings reared in culture pond. High concentrations of these compounds can be extremely damaging and cause massive mortalities of fingerlings. Water quality parameters such as temperature and pH were measured. The experiment was carried out for two months in which fish reared in each happas with stocking density of 75 fingerlings were fed with control diet (P_0-treatment), feed fortified with probiotic *Saccharomyces cerevisiae* at a dosage of (1.5×10^8 CFU/ml) with stocking density of 75 fingerlings (P_1-treatment), feed fortified with probiotic *Lactobacillus fermentum* at a dosage of (1.5×10^8 CFU/ml) with stocking density of 75 fingerlings (P_2-treatment) and feed fortified with probiotics *Saccharomyces cerevisiae* and *Lactobacillus fermentum* at a dosage of (1.5×10^8 CFU/ml) with stocking density of 75 fingerlings (P_3-treatment). In pre-treatment test with probiotics supplemented diet, six fingerlings were sample was taken and brought to veterinary medicine microbiological laboratory for Gastro Intestinal Tract (GIT) microbial investigation *via* dissecting their gut. Lactic acid bacteria and yeasts were isolated following the methods described by Kimaryo, et al. and Zapata and Lara-Flores [2,3]. The frequency of feeding was four times per day in pelletized form for the consecutive two months of the experimental period. Feeding allowance was adjusted in accordance with increase in body weight as recommended by Hogendoorn and the amount of feed delivered varied fortnightly (either increased or decreased) based on the weight gain of the fingerlings [4]. The overall research procedure was approved by the research committee of Hawassa University and thus all applicable international and national guidelines for the care of animals and use of animals were followed by the authors.

Feed formulation and methods used to fortify probiotics in pelleted diet

The basal diet was formulated to meet the nutritional requirement of Nile tilapia fingerlings. During feed formulation, fish carcass (fish meal) was the highest inclusion chosen for its high protein potency and good amino acid profile and therefore to meet the fingerlings high protein requirement. A basal diet contains bone meal 2.6 Kg (24.6%), soybean 3.3 Kg (31.3%), maize flour 2.2 kg (20.8%), and wheat flour 2.2 kg (20.8%), vegetable oil 0.24 kg (2.5%), having 30% crude protein was prepared. The basal diet had no probiotic additives, while the remaining feed were treated with probiotics *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and their combinations according to standard dose McFarland at cell density of 1.5×10^8 CFU/ml cells turbidity standard at 0.5 MFU on the McFarland scale. The pellet was produced at 1.5 mm pellet size and fed for fish stocked in happas for two months [5].

Fish sampling

Fish were sampled monthly using a clean bucket. All the fish were collected from each happas for individual weight and length measurements. Fish were weighed with a digital balance (0.1 g) (model SF-400A, Germany) and total length was measured using a measuring board (0.1 cm). Fish were returned to their respective happas after measurements. By the end of the experimental period, fish were deprived of feed for 24 hours all the fish were harvested, counted and weighed individually. The fish growth performances under different treatments were evaluated in terms of final total length (cm), final weight (g), Daily Weight Gain (DWG, $gday^{-1}$), body weight gain, Specific Growth Rate (SGR, % day^{-1}), survival rate (%) and Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER).

Feed utilization

The feed utilization can be measured interims of feed conversion ratio and protein efficiency ratio. They were calculated by the formula [6]:

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

$$PER = \frac{\text{Weight gain per fish (g)}}{\text{Protein intake (g)}} \times 100$$

Growth performance parameters

The growth performance of fish interims of final body weight, weight gain, daily growth rate and specific growth rate were calculated using the following formula:

Weight Gain (WG)=Mean final weight (gm)–Mean intial weight (gm)

$$\text{Daily Growth Rate (DGR)} = \frac{\text{Mean final weight (gm)} - \text{Mean intial weight (gm)}}{\text{Mean intial body weight gain}} \times 100$$

$$\text{Specific Growth Rate (SGR) \%/day} = \frac{(\text{LnWT} - \text{LnWt.})}{T - t} \times 100$$

Where:

SGR%=Percentage increase in body weight per fish per day

LnWT=Natural log of weight at time T

LnWt.=Natural log of initial weight

T=Time

t=Initial time

Ln=Natural logarithm

Survival rate of the fingerlings was determined after final harvesting of the fingerlings. The total number of fingerlings harvested was counted and then it was computed as:

$$\text{Survival rate (\%)} = \frac{\text{Number of survivals at the end of the experiment}}{\text{Number of fingerlings stocked}} \times 100$$

Analysis and identification of gut microbiota in pre-treatment with probiotics

At the end of the feeding period, fish were starved for 24 hours to allow gut evacuation and a random sample of 3 fish were taken from each treatment. Fish were sacrificed by icing, dissected and longitudinally opened. The entire fish intestine was aseptically removed. From each happas six fingerlings were collected randomly and brought laboratory for gut microbial investigation. Each parts of inoculum samples were transferred to liquid media of Brain Heart Infusion (BHI) broth. Broth containing inoculum samples were incubated at 37°C for 24 hours.

To investigate the presence of photogenic microorganisms from fish gut buffered peptone water was prepared at 1:9 ratio. In sterilized six test tubes 9 ml of buffered peptone water was added and 1 ml of microbial sample from the broth media was added in each test tubes. For investigation of *Salmonella* Rappaport Vacillidius Medias were prepared and 0.1 ml sample from buffered peptone water to the media was transferred and incubated at 42°C for 24 hours. The homogenate that was serially diluted to 10^{-1}-10^{-6} for bacterial and fungal analysis respectively and XLD-media for isolation of *Salmonella* and *Shigella* in appropriate measurement was prepared and loop full of bacteria samples from Rappaport Vacillidius media was transferred by inoculation needle streaked to Xylose Lysine Deoxycholate agar media (XLD and incubated at 37°C for 24 hours) [7].

Water quality monitoring and analysis

Water quality parameters *i.e.,* temperature, Dissolved Oxygen (DO), pH, total Ammonia (NH_3), were determined for the duration of the experiment with the same stocking density of fingerlings of Nile tilapia (*O. niloticus*) were measured *in situ* at two times a week using portable eco-checker multi-parameter water quality measuring instrument, made in the USA. Temperature (°C), dissolved oxygen (mgL^{-1}) and pH, were measured *in situ* using a multi-parameter water quality meter model number H19828 (Hanna Instruments Ltd., Chicago, USA). Water samples from each treatment happas were analyzed for total ammonium nitrogen (NH_3 mgL^{-1}), (DO mgL^{-1}) and pH using standard methods by Boyd and Tucker [8].

Analysis and identification of gut microbiota post-treatments with probiotics

Fishes were externally disinfected with 96.6-99% alcohol, and they were dissected and longitudinally opened. The three part of gut samples were transferred in to brain heart infusion broth using an inoculation needle and incubated at 37°C for 24 hours. Dominant bacterial and *Saccharomyces cerevisiae* from the cultures were purified and identified based on morphological characteristics and growth parameters using biochemical tests and standard techniques for isolating *Lactobacillus fermentum* and *Saccharomyces cerevisiae* [9].

Data analysis

One-way Analysis of Variance (ANOVA) at $P \leq 0.05$ was used to test the probiotic effect on the growth performance of the fingerlings. Analyses were carried out with Statistical Package for Social Science (SPSS version 21). All data were expressed as means ± Standard Error of the Mean (SEM).

Results

Growth performance parameters and feed utilization of Nile tilapia (*O. niloticus*

Nile tilapia (*O. niloticus*) fingerlings fed with control diet, diet supplemented with *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and their combinations at a dosage of (1.5×10^8 CFU/ml) revealed a significant increase ($p \leq 0.05$) in the Specific Growth Rate (SGR), Daily Growth Rate (DGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) as compared tofish fed with control diet. BWG, IBW, IBL, FBW, FBL and SR of the fish did not show any significant difference ($p \geq 0.05$) among treatment groups (Table 1).

Growth parameters	Treatments			
	P_0	P_1	P_2	P_3
IBW (g)	11.43 ± 1.27^a	10.20 ± 1.23^a	10.40 ± .67^a	11.23 ± 0.38^a
IBL (cm)	6.27 ± 0 .84^a	5.57 ± 1.58^a	5.87 ± 0.35^a	5.60 ± 0.66^a
FBW (g)	11.23 ± 2.58^a	39.47 ± .75^a	40.77 ± 4.07^a	40.60 ± 0.36^a
FBL (cm)	11.17 ± 0.19^a	11.77 ± .35^a	12.13 ± 0.32^a	11.67 ± 0.20^a
BWG (g)	19.83 ± 2.23^a	29.27 ± 1.35^b	33.43 ± 1.65^b	29.37 ± 0.29^b
SGR (%/day)	1.69 ± 0.17^a	2.28 ± 0.21^a	2.26 ± .06^a	2.14 ± 0.05^a
DGR (g/day)	0.33 ± 0.04^a	0.49 ± 0.02^{abc}	0.51 ± 0.05^c	0.49 ± 0.01^{abc}
FCR	3.52 ± 0.39^a	2.12 ± 0.09^b	1.89 ± 0.10^b	2.24 ± 0.02^b

PER	0.97 ± 0.11[a]	1.58 ± 0.07[b]	1.78 ± 0.09[b]	1.49 ± 0.01[b]
SR (%)	94.67 ± 2.67[a]	97.33 ± 2.67[a]	94.67 ± 2.67[a]	97.33 ± 2.67[a]

NB: Mean values (± SE) Means in the same rows sharing the same subscripts did not show significant difference (p ≥ 0.05). P_0: Control diet treatment; P_1: Control diet and *S. cerevisiae*; P_2: Control diet and *L. fermentum*; P_3: Control diet with *S. cerevisiae* and *L. fermentum*; IBW: Initial Body Weight; IBL: Initial Body Length; FBW: Final Body Weight; FBL: Final Body Length; BWG: Body Weight Gain; SGR: Specific Growth Rate; DGR: Daily Growth Rate; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio; SR: Survival Rate

Table 1: Growth performance parameters mean values (± SE) of Nile tilapia (O. niloticus).

Water quality parameters

The mean value of some water quality parameters such as temperature, pH, dissolved oxygen and ammonia were determined for the duration of experimental period was presented in Table 2. The mean values for water quality parameters during the experiment were determined and were ranged from 26.03-26.70°C for water temperature, 4.70-4.80 mgL^{-1} for dissolved oxygen, 7.86-8.03 for pH, 0.02-0.03 mgL^{-1} for total ammonia (Table 2). All the water quality parameters were optimal for fish growth performance.

Water quality parameters	Treatments			
	P_0	P_1	P_2	P_3
Temperature (°C)	26.20 ± 0.30[a]	26.70 ± 0.97[a]	26.31 ± 0.91[a]	26.03 ± 0.78[a]
Hydrogen ion concentration (pH)	7.96 ± 0.04[a]	8.03 ± 0.02[a]	7.96 ± 0.15[a]	7.86 ± 0.08[a]
Dissolved oxygen (mgL^{-1})	4.71 ± 0.09[a]	4.71 ± 0.04[a]	4.70 ± 0.06[a]	4.80 ± 0.01[a]
Ammonia (mgL^{-1})	0.03 ± 0.014[a]	0.03 ± 0.003[a]	0.02 ± .011[a]	0.03 ± 0.012[a]

NB: The values with same later are not significant difference (P>0.05) among treatment.

Table 2: Mean values of some water quality parameters were measured during the experiment (± SEM).

Fish gut microbial contents

The results of isolation of bacterial organisms from Nile tilapia (*O. niloticus*) fingerlings Gastro-Intestinal Tract (GIT) pre and post supplementation of diets with probiotics confirmed the existence of bacterial colonies with different morphological characters on different solid media and presented in Tables 3 and 4. This finding of the study revealed the detection of pathogenic microbial colonies. The pure colonies were identified by their different biochemical criterion which indicated the appearance of different bacterial flora. The results of bacterial identification in pre-treatment with probiotics showed the appearance of *E. coli*, *Salmonella* spp., *Shigella* species, and *Proteius mirabilus*. On the other hand the intestinal bacterial flora post treatment with probiotics revealed the same organisms of the pre-treatment in Nile tilapia (*O. niloticus*) treated with control diet. While those treated with diet supplemented *Saccharomyces cerevisiae* and *Lactobacillus fermentum* pathogens were not detected. Post-using probiotics in aquaculture pathogenic bacterial flora such as *E. coli*, *Salmonella* and *Shigella* were disappeared in re-isolation after dissecting fish gut during investigation. Intestinal bacterial flora post treatment with probiotics resulted in the dominancy of *Saccharomyces cerevisiae* and *Lactobacillus fermentum* in the gut of fingerlings treated with diets supplemented with these probiotics. Probiotics fortified at an inclusion level of (1.5×10^8 CFU/ml) dominates pathogens detected in pre-treatment tests. This study confirmed the effectiveness of probiotics in Nile tilapia (*O. niloticus*) reared in pond culture in to happas.

Parameters	FS-1	FS-2	FS-3
Total plate count	5.04 ± 0.01[a]	5.16 ± 0.07	5.27 ± 0.03
Salmonella	Nd	1.56 ± 1.56[a]	Nd
E. coli	4.41 ± .17[a]	4.78 ± .07[a]	4.90 ± .01[a]
Shigella flexneri	4.76 ± .26[a]	4.35 ± .11[a]	3.06 ± 1.54[a]
Proteius mirabilus	Nd	4.45 ± 0.17[a]	4.91 ± 0.23[a]
Candida albicans	4.94 ± 0.06[a]	Nd	4.93 ± 0.25[a]

NB: Rows sharing the same subscripts did not show significant difference (p ≥ 0.05). FS-1: Fish sample one; FS-2: Fish sample two; FS-3: Fish sample three; Nd: Not detected

Table 3: Table of gut microbial contents in pretreatment test.

	P_0	P_1	P_2	P_3
Total plate count	6.95 ± 0.06^{b}	6.98 ± 0.10^{b}	6.06 ± 0.05^{b}	6.98 ± 0.05^{b}
S. cerviciae	ND^{a}	2.46 ± 0.09^{b}	2.89 ± 0.03^{d}	3.90 ± 0.06^{f}
L. fermentum	ND^{a}	2.51 ± 0.14^{c}	3.98 ± 0.14^{e}	3.33 ± 0.27^{g}

Table 4: Gut microbiota of Nile tilapia fed on *S. cerevisiae* and *L. fermentum* treated feed in the happas of concrete ponds for 2 months. (SEM ±) (Log CFU/ml) (10^{-4}).

Discussion

Growth performance and feed utilization

This study examined the effects of diet fortified with *Lactobacillus fermentum*, *Saccharomyces cerevisiae*, and their combinations on growth performance of Nile tilapia (*O. niloticus*) fingerlings. Feed supplemented with probiotics *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and their combinations at dosage of level of (1.5×10^{8} CFU/ml) resulted in significantly increase growth performance SGR, and DGR of Nile tilapia (*O. niloticus*) fingerlings than fed control diet. This could be attributed improved nutrient digestibility and availability to the fish. This study confirmed that an improved growth rate was due to the presence of growth-stimulant components that existed in selected probiotic microorganisms in aquaculture of Nile tilapia. The result of the present study was agreed with Lara-Flores, et al. who reported that Nile tilapia (*O. niloticus*) fed diets containing a mixture of bacterial (*Streptococcus faecium* and *Lactobacillus acidophilus*) and the yeast (*Saccharomyces cerevisiae*) promoted growth performance of the fish [10]. According to previous finding indicated that the highest Specific Growth Rate (SGR) was recorded in fish treated with diet containing *Saccharomyces cerevisiae* was (2.30%), whereas the lowest SGR was recorded in a control diet of treatment (1.73%). This study was consistent with previous findings reported by Rahman, et al., and Chen, et al. [11,12].

The mean daily growth rate of the current study was highest in Nile tilapia (*O. niloticus*) fingerlings fed diet supplemented with *Lactobacillus fermentum* whereas the lowest mean daily growth was recorded in Nile tilapia (*O. niloticus*) fingerlings fed control diet. This might be recognized to conducive environmental situation, standardize quality of formulated diet, the type of feed ingredient and dosage of probiotics inclusion level. This variation might be the ability of *Lactobacillus fermentum* better nutrient digestion due to different enzymatic activities in the host gut which lead to facilitated daily growth rate of fish. The application of probiotics in diets results in more nutrient digestibility for feed stuffs. Similarly, Hasan, et al. reported that mean daily growth rate obtained using commercial feed and commercial probiotics revealed the highest daily growth rate [13].

The findings of this study showed that probiotics *Lactobacillus fermentum* and *Saccharomyces cerevisiae* strains significantly improved the feed utilization efficiency (FCR and PER) of Nile tilapia (*O. niloticus*) fingerlings provided in feed separately and in combinations with (*Lactobacillus fermentum* and *Saccharomyces cerevisiae*), supplemented with diet. This might be probiotics improved digestion and nutrient absorption of feed by producing digestive enzymes that can alter their gut environment. According to Merrifield, et al. and Welker and Lim, reported that probiotics consumption enhanced the fish appetite, boost organisms feed digestibility *via* stimulating digestive enzymes and maintaining the balance of intestinal pathogenic micro flora [14,15].

In line with the present study, Mesalhy, et al. reported that higher growth rate was detected in fish fed with probiotic-supplemented diets than those fed on the control diet [16-18].

In this study statistical analysis of variance showed that a significant increase in feed conversion ratio. The highest FCR was recorded in fish fed diet containing *Lactobacillus fermentum* (1.89) whereas the lowest FCR was recorded in fish fed diet containing control diet treatment that had a value of (3.52). The best Food Conversion Ratio (FCR) values were indicated that higher muscle building in their body with low amount of feed (g). Feed Conversion Ratio (FCR) is to assess feed utilization and absorption, which is the ability to convert feed to their flesh. The previous finding as reported by Akanmu, et al. on African cat fish (*Bidorsalis*) juveniles was disagreed with current finding on Nile tilapia (*O. niloticus*) fingerlings the highest FCR value was recorded in fish fed with diet containing *Lactobacillus fermentum* was (1.26) and where as a lowest FCR was recorded in fish fed with diet containing *Saccharomyces cerevisiae* and *Lactobacillus fermentum* were (1.96) it might be species variation among Nile tilapia (*O. niloticus*) fingerlings and African cat fish (*Bidorsalis*) juveniles. The improvement of feed utilization for fish diet supplemented with probiotics, might be improved the intestinal microbial flora balance which in turn lead to better feed absorption quality, increased enzymatic activities, more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids [19]. The PER results indicated that supplementing diets fortified with probiotics significantly improved protein utilization in Nile tilapia (*O. niloticus*) fingerlings. The protein efficiency ratio indicated that utilization of dietary protein using the gain of biomass. This contributed to optimizing protein use for growth which is the most expensive feed nutrient. The current study on protein efficiency ratio value was consistent with previous findings as reported by Akanmu, et al. with result of (1.62). The highest PER was recorded in treatment group received diet supplemented with *Saccharomyces cerevisiae* it had PER value of (1.97) whereas the lowest PER was recorded in diet supplemented with *Saccharomyces cerevisiae* and *Lactobacillus fermentum* with a value of (1.27) [20].

Gut microbial content post treatment with probiotics

The results of microbial profile of the different parts of the intestinal tract anterior, middle and posterior parts in pre-treatment with control diet revealed the presence of many pathogenic bacteria existed in the gut of sampled fish. The results of bacterial identification in pre-treatment investigations revealed the existence of some members of Enteriobacteriaceae species namely *E. coli*, *Salmonella*, *Shigella*, *Proteius mirabilus* were detected through biochemical identification of the bacterial organisms from Nile tilapia (*O. niloticus*) reared in pond culture.

The bacteriological profile of the different parts of the intestinal tract of Nile tilapia (*O. niloticus*) treated with probiotics *Saccharomyces cerevisiae* and *Lactobacillus fermentum* showed more or less different profile concerning disappearance of some members of *Enteriobacteriaceae*.

Nile tilapia (*O. niloticus*) that fed control diet has been infected by Enteriobacteriaceae family namely *E. coli* (motile-form), *E. coli* (non-motile form), *Salmonella*. *C. albicans* and *Shigella* appearance were proved by biochemical reaction test. From treatment group received diet supplemented with probiotics *Saccharomyces cerevisiae* and *Lactobacillus fermentum* fingerlings, the probiotics were re-isolated again post-treatment. This illustrated that the gastrointestinal tract of Nile tilapia (*O. niloticus*) fingerlings were completely dominated with treated probiotics. Nile tilapia (*O. niloticus*) fingerlings in the treatments with *Lactobacillus fermentum* (P_2) fortified diets were not detected the existence of *E. coli*, *Salmonella*, *Shigella* and *P. mirabilus*.

The finding of microbial test of the gut of Nile tilapia (*O. niloticus*) in the four treatments designated the colonizing ability of *Saccharomyces cerevisiae* and *Lactobacillus fermentum* in the gut. This could be attributed to the capacity of these probiotics to bind the intestinal mucosal cell receptors for immune-stimulation, improved disease resistance for some members of *Enteriobacteriaceae* and fungi strains, reduced stress response, and improved gastro-intestinal morphology. Moreover, it provides significant benefit to fish farmer as well as consumer.

Probiotics are also referred to as bio-proteins that comprising living microbial cells that augment the colonization and composition of the growth and gut micro flora in animals and stimulate digestive processes and immunity. Probiotics may minimize the incidence of diseases or lessen the severity of outbreaks in aquaculture. It serves as alternative to microbial chemotherapeutics ability. Probiotics are primarily used as feed additives to prevent infectious intestinal diseases through the secretion of micro-toxins that inhibit the growth of other virulent micro-organism (such as *Escherichia coli* and *Salmonella*) in the intestinal lumen of the host.

Conclusion

This investigation concluded that *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and the combination of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* at dosage of 1.5×10^8 CFU/mL increased growth performance of fish and enhanced the stimulation of non-specific immunity to fish pathogens like *Salmonella enterica*, *Shigella flexineri*, *E. coli*, *C. albicans* and *P. mirabilus*. Administration of *Lactobacillus fermentum* probiotic enhanced the activity of different intestinal enzyme which could contributed to the efficient digestion of feed in fish gut.

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Conflict of Interest

The authors declare no conflict of interest.

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