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Article

Application of Probiotics in Aquaculture to Improve Growth and Health Performances of Nile tilapia

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Abstract: This research was aimed to apply probiotics in aquaculture to improve fish health and growth performances of Nile tilapia. Fingerlings with a mean initial body weight of 11.43±1.27 and mean initial body length of 6.2±0.84 were stocked in 12 happas. Fish were fed four times daily with four different diets having 30% crude protein at 5% of their body weight and reared for two months. The four diets were control diet, diets treated with *Saccharomyces cervisiae*, *Lactobacillus fermentum* and their combinations at a dosage of (1.5×10^{8}CFU/ml). Growth performance and gut pathogenic microbial of fish were investigated. Accordingly results indicated that the highest performance interims of body weight gain (33.43±1.65g), daily growth rate (0.51±0.05g), feed conversion ratio (1.89±0.10), and protein efficiency ratio (1.78±0.09) was achieved in fish fed with *L. fermentum* whereas, the lowest performance (19.83±2.23, 0.33±0.04, 3.52±0.39, and 0.97±0.11) respectively was achieved in fish fed with control diet. Salmonella, Shigella, *E. coli* and, Proteus were detected in gut of fish. In conclusion, the present study confirmed that Nile tilapia fed with probiotics *Lactobacillus fermentum* and *Saccharomyces cerevisiae* based diets improved growth and health of Nile tilapia reared under pond culture condition.

Keywords: Aquaculture; gut micro flora; health; Nile tilapia; probiotics

1. Introduction

Ethiopia is one of the developing countries where food security is not ensured yet and there is a need to expand aquaculture, particularly among those rural areas where there is a high shortage of proteinaceous foods. However, the growth of aquaculture sector is greatly impeded due to low growth performance fish reared under different production systems. In other hand, disease outbreaks by diverse groups of pathogenic bacteria could lead high mortality while the production systems are intensified. Environmental eco-friendly alternative method of using probiotics to improves the quality and sustainability of the aquaculture production sector [27] widely practiced in other countries is yet to be practiced in Ethiopia. Diseases especially bacterial infections remain the primary constraints to continue expansion of aquaculture. Probiotics are being recognized as an alternative therapy for health management due to restrictions on antibiotics and limitations of vaccinations and chemotherapy [25]. But the use of probiotics in aquaculture is new when viewed in comparisons with farm animal feed supplements such as in pig, cattle and poultry nutrition from the 1970's, [32].

It is now well established that probiotics play a vital role in maintaining gut health and growth performance by modulation of microbial community structure [23]. The rapid evolution of probiotics in aquaculture is well established due to the adverse effects of widely used antibiotics and broad-spectrum chemicals which kill most of the beneficial bacteria along with the pathogenic bacteria to the aquatic species [15].

Therefore, enhancement of the immune system of reared fish and establishment of normal gut microbiota is vital, as it affects a wide range of biological processes including; nutrient utilization, development and assembly of gut associate lymphoid tissue (GALT) and the ability to fight infections. The use of probiotics is gaining acceptance in fish farming as a means of improving growth and health performance of fish.

2. Materials and Methods

2.1. 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 275km 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 1714m.a.s.l.

2.2. Study design

Nile tilapia fingerlings used in this experiment were collected from CARE. Three hundred (300) Nile tilapia fingerlings were collected and 25 fingerlings were stocked in 12 hapas. The fingerlings were stocked with a mean initial body weight of 10.41±0.76g and mean body length of 5.87±0.85cm. Fingerlings were acclimatized in the happas for 15 days before conducting an 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). Water was changed once a week to maintain good water quality by removing wastes. Water quality parameters such as temperature and pH were measured using thermometer and pH-meter respectively. The experiment was carried out for two months in which the fish found in each of the hapa were fed with control diet (P0), feed fortified with probiotic *S. cerevisiae* (P_1), feed fortified with probiotic *L. fermentum* (P_2-) and feed fortified with probiotics *S. cerevisiae and L. fermentum* (P_3). Prior to the start of feeding of probiotics fortified with diets six fingerlings per hapa were collected for gastro intestinal tract (GIT) microbial analysis.

2.3. Identification of lactic acid bacteria and Yeasts

Lactic acid bacteria and yeasts were isolated following the methods described by [14,16].

2.4. 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. 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 0.24kg (2.5%), having 30% crude protein was prepared. The basal diet had no probiotic additives, while the remaining feed were treated with probiotics *Lactobacillus fermentum*, *S. cerevisiae* and their combinations according to standard dose McFarland at cell density of 1.5×10^8CFU/ml cells turbidity Standard at 0.5MFU on the McFarland scale. The pellet was produced at 1.5mm pellet size and fed for fish stocked in hapa for two months.

2.5. Fish sampling

Fish were sampled monthly using a clean bucket. All the fish were collected from each hapa for individual weight and length measurements. Fish were weighed with a digital balance (0.1g) (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. At 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

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(cm), final weight (g), daily weight gain (DWG, g day^{-1}), body weight gain, specific growth rate (SGR, % day^{-1}), survival rate (%) and feed conversion ratio (FCR) and protein efficiency ratio.

2.6. Feed utilization

The feed utilization can be measured in terms of feed conversion ratio and protein efficiency ratio. They were calculated by the formula;

$$\text{FCR} = \frac{\textbf{Total feed consumed by fish(g)}}{\textbf{Total weight gain by fish(g)}}$$ as computed by [20]

$$\text{PER} = \frac{\textbf{Weight gain per fish(g)}}{\textbf{Protein intake (g)}} \text{ X } 100$$ as computed by [20]

2.7. Growth parameters

The growth performance of fish in terms of final body weight, Wight gain, gaily growth rate and specific growth rate were calculated using the following formula:

$$\text{Weight Gain (WG)} = \textbf{Mean Final Weight(gm)} - \textbf{Mean Intial Weight(gm)}$$

$$\text{Daily Growth Rate (DGR)} = \frac{\textbf{Mean Final Weight(gm)} - \textbf{Mean Intial Weight(gm)}}{\textbf{Mean Intial Body Weight Gain}} \times \mathbf{100}$$

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

Where: SGR%=Percentage increase in body weight per fish per day. Ln WT=natural log of weight at time T, LnWt. = natural log of initial weight, T=time T, t=initial time, Ln=Natural Logarithm. Where: 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:

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

2.8. Analysis and identification of gut microbiota in pretreatment test with probiotics

From each happas two fish were collected randomly and brought to veterinary medicine microbiological laboratory for gut microbial content analysis. Each parts of inoculum samples were transferred to liquid media of brain heart infusion broth (BHI). Broth containing inoculum samples were incubated at 37^{o}c for 24 hours.

To investigate the presence of photogenic microorganisms from fish gut buffered peptone water was prepared at a 1:9 ratio. In sterilized six test tubes 9-ml of buffered peptone water was added and 1ml of microbial sample from the broth media was added in each test tubes. For investigation of *Salmonella* Rappaport *vacillidius* Medias were prepared and 0.1ml sample from buffered peptone water to the media was transferred and incubated at 42^{0}c for 24hr. 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 XLD- media and incubated at 37^{0}c for 24hours.

2.9. 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 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 (mg L^{-1}) and pH, were measured *in situ* using a multi-parameter water quality meter model number H19828

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(Hanna Instruments Ltd., Chicago, USA). Water samples from each treatment happas were analyzed for total ammonium nitrogen (NH_3- mg L^{-1}), (DO- mg L^{-1}) and pH using standard methods.

2.10. Analysis and identification of gut microbiota post-treatments with probiotics

At the end of the feeding period, fish were starved for 24 hours to allow gut evacuation and a random sample of 6 fish was taken from each treatment group. Fishes were externally disinfected with 96.6-99% alcohol, and they were dissected and longitudinally opened. The entire fish intestine was aseptically removed and divided into three parts (anterior, middle, and posterior) in the per-treatment test. 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 yeast colonies from the cultures were purified and identified based on morphological characteristics and growth parameters using biochemical tests and standard techniques for isolating Bacillus spp. and yeast [22].

2.11. Data analysis

One-way Analysis of Variance (ANOVA) at P= 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).

3. Results

3.1. Growth Performance Parameters of Fishes

Final body weight, final body length, body weight gain, specific growth rate, daily growth rate, feed conversion ratio, and protein efficiency ratio (PER) of the experimental fish after the feeding experiment are presented in Table 1. The growth performance and feed utilization efficiency of fish fed with *L. fermentum* followed by combination of *L. fermentum and S. cerevisiae and S. cerevisiae* revealed a significant increase ($p \leq 0.05$) in the body weight gain (BWG), Feed conversion ratio (FCR), protein efficiency ratio (PER) and daily growth rate (DGR) compared with fish fed with control diet. Daily growth rate and survival rate of the fish did not show a significant difference ($p \geq 0.05$) among treatment groups (Table 1).

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

Growth parameters	Treatments			
	P0	P1	P2	P3
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})	1.69±0.17^a	2.28±0.21^a	2.26±.06^a	2.14±0.05^a
DGR(g/day^{-1})	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

Note: Mean values (±SE) Means in the same rows sharing the same subscripts did not show significant difference ($p \geq 0.05$). P^0= (control diet treatment), P_1= (control diet and S. cerevisiae), P_2= (control diet and fermentum), P_3= (control diet with S. cerevisiae and 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).

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3.2. 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^{0}C for water temperature, 4.70- 4.80 mg L^{-1} for dissolved oxygen, 7.86- 8.03 for pH, 0.02-0.03mg L^{-1} for total ammonia (Table 2). All the water quality parameters were optimal for fish growth performance.

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

	Treatments			
Water quality parameters	**P0**	**P1**	**P2**	**P3**
Temperature (^{o}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 (mg L^{-1})	4.71±0.09 ^{a}	4.71±0.04 ^{a}	4.70±0.06 ^{a}	4.80±0.01^a
Ammonia (mg L^{-1})	0.03±0.014^a	0.03±0.003^a	0.02±.011^a	0.03±0.012^a

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

3.3. Fish gut microbial contents

The results of isolation, of bacterial organisms from Nile tlapia fingerlings gastro-intestinal tract (GIT) pre and post application of diets supplemented with probiotics confirmed the presence of colonies with different morphological characters on different solid media and presented in Tables 3 and 4. The results of isolation of bacterial from intestinal tract in pretreatment test revealed the presence of pathogenic colonies with different morphological characters on different solid media. The pure colonies were identified by their different biochemical criterion which indicated the appearance of different bacterial flora. The results of bacterial identification pre-treatment 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 received control diet while in those received S. Cerevisiae and L.fermentum were observed. Pathogenic bacterial flora post-using probiotics in aquaculture showed disappearance of E.coli, Salmonella spp, Shigella and in re-isolution after dissecting fish gut in laboratory investigation.

Intestinal bacterial flora of post treatment with probiotics resulted in the appearance of Saccharomyces Cerevisiae and Lactobacillus fermentum received during experimental period. Probiotic fortified at an inclusion level of (1.5×10^8CFU/ml) dominates pathogens detected in pretreatment tests. The current study confirmed the effectivines of probiotics in Nile tilapia rearing aquaculture pond in to happas.

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

Parameters	**FS-2**	**FS-4**	**FS-6**
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

Note: 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), and Nd (Not detected).

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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}).

	P0	P1	P2	P3
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]

4. Discussion

4.1. Growth Performance and Feed utilization

This study examined the effects of diet fortified with *L. fermentum*, *S. cerevisiae*, and their combinations on growth performance of Nile tilapia fingerlings. Feed supplementation with probiotics (*S. cerevisiae*, *L. fermentum* and their combinations (P3)) resulted in significantly higher growth performance (SGR, DGR and BWG) of the fish on Nile tilapia fingerlings over the control diet. This could be attributed to improved nutrient digestibility and availability to the fish. This study also 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 [16] who reported that Nile tilapia fed diets containing a mixture of bacterial (*Streptococcus faecium* and *Lactobacillus acidophilus)* and the yeast (*Saccharomyces cerevisiae)* promoted growth performance of the fish. 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). Comparable previous findings were also reported by [5,26]. The current study was also in agreement as reported by [9].

The mean daily growth rate of the current study was highest in fish received *L. fermentum* whereas the lowest mean daily growth was recorded in 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 in fish diet. This variation of the current study was also might be due to the ability of *L. fermentum* better nutrient digestion due to different enzymatic activities in the host gut which lead to facilitated daily growth rate of fish. As also reported by [15] suggested that application of probiotics in diets results in more nutrient digestibility for feed stuffs. Similarly, [11] reported that mean daily growth rate obtained using commercial feed and commercial probiotics showed that the highest daily growth rate of Nile tilapia fingerlings.

The findings of this study showed that probiotics *L. fermentum* and *S. cerevisiae* strains in aquaculture significantly ($p \leq 0.05$) improved the feed utilization efficiency (FCR and PER) of fish when provided in feed separately and in combinations (*L. fermentum* and *S. cerevisiae*). as the same time feed fortified with control diet. This could be explained by the ability of probiotics to improve digestion and nutrient absorption of fish feed by producing digestive enzymes that can alter the gut environment. According to [18,36], report probiotics consumption has been developed to enhance the fish appetite, boost organisms feed digestibility by stimulating the excretion of digestive enzymes and maintaining the balance of intestinal pathogenic micro flora. In line with the present study, [19] reported that higher growth rate was detected in fish fed with probiotic-supplemented diets than those fed on the control diet. This study was agreed as reported by [9,17,21,29,31,33,38].

In this study statistical analysis of variance showed that a significant increase ($p \leq 0.05$) in feed conversion ratio. The highest FCR was recorded in fish fed diet containing *L. 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 the flesh. The previous finding as reported by [6] on African cat fish *(Bidorsalis*) juveniles was agreed with current finding on Nile tilapia (*O. niloticus*) fingerlings the highest FCR value was recorded in fish fed with diet containing *L.*

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fermentum was (1.26) and where as a lowest FCR was recorded in fish fed with diet containing *Saccharomyces cerevisiae* and *L. fermentum* were (1.96).

The improvement of feed utilization for fish, supplemented with probiotics, could be exhibited to improvement in the intestinal microbial flora balance which, in turn, lead to better absorption quality, increased enzyme activities [16,35], and more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids [16]. The PER results indicated that supplementing diets fortified with probiotics significantly ($p \leq 0.05$) improved protein utilization in Nile tilapia (*O. niloticus*) fingerlings. The Protein efficiency ratio indicates the utilization of dietary protein using the gain of biomass. This contributes to optimizing protein use for growth which is the most expensive feed nutrient. The current study on protein efficiency ratio value was comparable with previous finding on PER as reported by [20]) (1.62) the highest PER was recorded in treatment received in *saccharomyces cerevisiae*, (1.97) whereas the lowest PER was recorded in *Saccharomyces cerevisiae* and *L. fermentum was* a value of (1.27).

Regarding to the water quality parameters, the values were within the acceptable ranges recommended for Nile tilapia (*O. niloticus*). However, according to Hassan *et al.* (2021) the optimum water temperature required for Nile tilapia is 25-27^{0}C, Dissolved oxygen content is 5mg/L and pH is 5.5-9.0. In aquaculture, probiotics could improve water quality by influencing the water born microbial population and by reducing the number of pathogens in the vicinity of farming species. From the fact that the bacterial profile of fish intestinal tract is usually a reflection of microbial organisms in the environment.

4.2. Gut microbial content post treatment with probiotics

The results of microbial profile of the different parts of the intestinal tract anterior, middle and posterior part of in pre-treatment with control diet revealed the presence of many pathogenic bacterial organisms in the gut of sampled fish. The results of bacterial identification in pre-treatment investigations revealed the existence of some members of *Enterobacteriaceae species* namely *E.coli*, *Salmonella*, *Shigella*, *Proteus mirabilis* were detected through biochemical identification of the bacterial organisms from Nile tilapia kept in aquaculture.

The bacteriological profile of the different parts of the intestinal tract of Nile tilapia gets a S. cerevisiae and *L. fermentum* shown more or less different profile concerning disappearance of some members of *Enterobacteriaceae*. Nile tilapia that feed control diet has been infected by Enterobacteriaceae 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 probiotics *S.cerevisiae* and *L. fermentum* supplemented diet feeder fingerlings, all of the probiotics were re-isolated. Nile tilapia (*O. niloticus*) in the treatments with *L. fermentum* (P2) fortified diets were not detected the existence of E. *coli* (motile form) *Salmonella, Shigella*, and *P. mirabilis*.

The finding of microbial test of the gut of Nile tilapia in the four treatments designated the fighting capability of *S. cerevisiae* and *L. fermentum*. This could be attributed to the capacity of these probiotics bacteria and yeast to bind the intestinal mucosal cell receptors for immune-stimulation, improved disease resistance for some members of *Enterobacteriaceae* and fungi strains, reduced stress response, improved gastro-intestinal morphology and benefits to the fish farmer or 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. As reported by [18], probiotics may minimize the incidence of diseases or lessen the severity of outbreaks in aquaculture or fish culture. It can be used 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.

It could be concluded that *Lactobacillus fermentum, Saccharomyces cerevisiae* and the combination of *Lactobacillus fermentum and Saccharomyces cerevisiae* at inclusion level 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 can enhance the activity of different intestinal enzyme which could contributed to the efficient digestion of feed in fish gut.

5. Conclusions

This study confirmed that Nile tilapia fed with fortified *Lactobacillus fermentum* at dosage of 1.5×10^8cfu/ml, *Saccharomyces cerevisiae* at 1.5×10^8cfu/ml and their combinations at 1.5×10^8 cfu/ml improved the growth performance parameters like body weight gain, feed conversion ratio, protein efficiency ratio, daily growth rate, and also improved survival rate, by reducing pathogenic microbial loads on tested fish over that of the control experimental fish. Therefore, it could be concluded that *Lactobacillus fermentum* at inclusion level (1.5×10^8 cfu/mL), *Saccharomyces cerevisiae* at (1.5×10^8 cfu/mL) and the combination (*Lactobacillus fermentum and Saccharomyces cerevisiae)* at (1.5×10^8 cfu/mL), triggered and enhanced the stimulation of non-specific immunity to fish pathogens like *salmonella enterica, Shigella flexineri, E. coli, C. albicans* and *P. mirabilus*. *Lactobacillus fermentum* bear significant ability of producing bacteriocins-like inhibitory substances which can contribute a lot to disease resistance. This means that they can overpower the growth of other pathogenic bacteria to inhibit certain diseases. Administration of *Lactobacillus fermentum* probiotic can enhance the activity of different intestinal enzyme which could contributed to the efficient digestion of feed in fish gut.

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