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Research Paper

Dietary Effects of *Saccharomyces cerevisiae* on Nile Tilapia (*Oreochromis niloticus*) Juveniles Challenged with *Aeromonas hydrophila*

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Abstract

A 14-week study was conducted to examine the dietary effects of Saccharomyces cerevisiae on growth performance, hematological response and, resistance of Oreochromis niloticus juveniles to Aeromonas hydrophila as a pathogenic organism. The study had 5 treatments and 3 replicates in a complete randomized design. The growth study which was run for 12 weeks, with the fish fed twice per day at 3% body weight. The experimental diets contained Saccharomyces cerevisiae at 0, 20, 25, 30 and 35 ml/kg, represented as T1 (control diet), T2, T3, T4 and T5, respectively. The study had growth parameters monitored, after which the fish were challenged with Aeromonas hydrophila through intramuscular injection and, kept for another 2 weeks to monitor the clinical signs and the mortality rate. The study revealed that diet T5 supported better fish growth and survival rates. All the challenged fish were sluggish after being challenged. The highest relative percentage survival was obtained in treatments T4 and T5, while T1, T2 and T3 had the least relative percentage survival. Higher values of hemoglobin packed cell volume, red blood cell, and white blood cells were recorded in the treated diets with the highest recorded in T5, while the least was obtained in T1. In conclusion, diet T5 was observed to support growth rate, improved blood constituents and increased the resistance of Nile Tilapia exposed to Aeromonas hydrophila. Therefore, diet containing S. cerevisiae at 35 ml/kg could be recommended to the fish farmers for the production of disease resistant and fast growth Oreochromis niloticus.

1. Introduction

Aquaculture development in the developed and developing countries cannot be under estimated for its relevance in food security and job creation leading to improvement in foreign earnings (Bene et al., 2016). Numerous aquatic organisms are being used as raw materials for some finished products, while some fish, like shell and fin, constitute an important part of human diet in many countries (FAO, 2022). Nile Tilapia is the most common species of Tilapia (Cichlids) and it has about 77.3 % global production increase from 1001.5 thousand tons in 2000 to 4407.2 thousand tons in 2020 (FAO, 2022). In Nigeria, Nile Tilapia is the second largest cultured fish after catfish.

The increase in production rate of *Oreochromis niloticus* in Nigeria is an indication that the aquaculture practice is enhancing its production, thus reducing the

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farmers' dependence on the wild stocks for Tilapia production. *O. niloticus* is the most accepted fish among the Tilapia species and it is considered as the food fish of great economic importance in Nigeria, after catfish (Oluwalola et al., 2020). Nile Tilapia has been reported to thrive successfully under different climatic weather conditions and culture systems. It grows bigger and faster than other Tilapia fish species; it could reproduce in a wide range of environmental conditions; and it could accept supplemental feed (Oluwalola et al., (2020). *O. niloticus* is also considered as the most important farmed Tilapia worldwide (Samaddar, 2022), making Tilapia and other cichlids to be ranked second in the importance of fin fish cultured worldwide after common carp (*Cyprinus carpio*) (FAO, 2023). With the advancement in technology, especially in the area of genetics, FAO (2018) has predicted a tremendous increase in production value of *O. niloticus* by 2030 over that of the 2010 production.

Despite these positive reports and characteristics, *O. niloticus* is yet to obtain its maximum aquaculture potentials in Nigeria due to the major draw-back of stunted growth leading to its low commercial value (Fashina-Bombata and Megbowon, 2012). The potential energy required for growth in *O. niloticus* is used for the formation and development of gonads, making them reproduce at the early stage of live, thus reducing their growth. Fish growth and disease resistance have also been reported to be of great concern in aquaculture, therefore adequate feed that could improve the growth rate and the health condition of the fish must be made available to the fish farmers in order to produce high commercial value fish (Akanmu et al., 2022).

Fish diseases have been a major hindrance to aquaculture development globally; however, its prevention has been directed towards the use of bio-friendly approaches like the use of probiotics. Several studies have been conducted on the use of probiotics in enhancing the growth rate and health of different fish species (Mamun et al., 2018). Probiotics have been described as microbial organisms that show beneficial effects on the health and wellbeing of the host animals. They are found harmful to pathogenic organisms, but not to their hosts. Numerous microorganisms, such as *Bacillus species, Lactobacillus species, Enterococcus species, Carnobacterium species,* and *Saccharomyces cerevisiae,* among others, have been reportedly administered to different fish species as probiotics to enhance their growth and to improve their health status. Probiotics are considered the promising biological control strategy and they are also an integral part of aquaculture practices for improving growth and disease resistance (Balcazar, 2006). They are also considered an alternative means of fish treatment to chemotherapy (Akanmu, 2018). Sharma et al. (2022) reported probiotics to improve fish culture water quality and to improve the immune system of a fish, thus reducing the disease outbreak. *Saccharomyces cerevisiae* is a microorganism of genus of yeast. It is robust yeast that is capable of withstanding stressful conditions and has a high fermentation efficiency, which multiplies rapidly at room temperature (Sharma et al., 2022). *Saccharomyces cerevisiae* was reported to enhance growth rate of *Amatitlania nigrifasciata* at 2% inclusion rate.

Culture of *O. niloticus* in Nigeria has not only been truncated by inability to get good and fast growing seeds, but also by inability to obtain seeds of improved immune system and the seeds that are able to adequately utilize their feeds. The dietary requirement of the cultured fish is probably one of the most important factors influencing fish farming, thus making the study of fish nutrition to embrace the use of potential biotechnology and bioengineering ingredients, feed additives and probiotics in fish production. Good diets should not necessarily meet the problem of hunger but, should also be adequately utilized to enhance their growth and supply every nutrient required. The blood components of a fish could be a perfect indicator of its well-being as related to its nutritional status (Esmaeili, 2021). There is, therefore, a need to consider the dietary effects of *S. cerevisiae* on the growth performance, blood constituents and disease resistance of Nile Tilapia (*O. niloticus*) fed diets containing different levels of *S. cerevisiae* and challenged with *Aeromonas hydrophila*.

2. Materials and Methods

2.1. Materials Used

A total of one hundred and eighty (180) juvenile *Clarias gariepinus* were procured from the fish farm of the Department of Fisheries and Aquatic Resources Management, Osun State University, Osogbo, Nigeria.

The plastic aquarium used for this study were procured from the market, while the commercial fish feed and the fish feed ingredients were purchased from a reputable fish feed merchant in Osogbo, Nigeria. The *Saccharomyces cerevisiae* was produced from the freshly tapped palm wine which was purchased from a palm wine tapper in Ibadan (Nigeria) and was immediately taken to the food laboratory of the Department of Microbiology, University of Ibadan, for onward process to form *S. cerevisiae*.

2.2. Experimental Design and Procedure

This study was conducted in the fish farm of the Department of Fisheries and Aquatic Resources Management, Osun State University, Nigeria. The experiment was carried out in 15 rectangular plastic tanks of 40 L capacity in a complete randomized design. Each plastic tank was stocked with 10 pieces of experimental fish in 35 L of water. The study which was designed to contain five treatments was replicated thrice. A total of 150 pieces of *O. niloticus* juvenile of average body weight 4.86±0.12 g were used for this study. The fish were acclimatized for 7 days (Jenkins et al., 2014) before the growth study, which lasted for 12 weeks. During the acclimatization, the fish were fed with commercial fish feed. At the start of the study, the initial average weights of the fish in each treatment were determined while, the subsequent increase in weight was monitored fortnightly by using a digital sensitive scale. The fish were fed 3% body weight at 2 rations (9.00 and 16.00 o'clock).

The fish were sampled for the new mean weight at every two weeks, using digital pocket scale of accuracy 0.1 gram (Sensitive Electronics, Rajkot, India). The water quality parameters were monitored using API freshwater master test kit 800 (Mars Fishcare Inc., USA) for nitrite, nitrate and ammonia determination. The water temperature and the pH were determined by using hand held pocket sized digital New pHep® (Hanna Instruments, USA), while the dissolved oxygen was monitored using Extech DO-meter (Industrial Electronics Inc., USA). The fish feed ingredients were milled using Honda fuel powered hammer mill (locally produced). The milled ingredients were manually but thoroughly mixed and transferred into the flat die pelletizer (Caps Feed Limited, Nigeria) situated in the fish feed mill section of the Department of Fisheries and Aquatic Resources Management, Osun State University, Nigeria. The diets were made in 2 mm size using the 2 mm dice of the machine.

2.3. Isolation and Identification of *S. cerevisiae*

The freshly tapped palm wine sample which was collected into a sterile plastic bottle with a screw cap was kept in a container with an ice pack and moved to the food laboratory of the Department of Microbiology, University for Ibadan for laboratory analysis. One ml of the palm wine sample was serially diluted from 10 to 10^{10}cfu/ml and centrifuged for 15 min. Fifty grams of yeast extract agar was weighed into the conical flask and dissolved in one litre of distilled water. The solution was autoclaved for 15 min at 121 ^{0}C and later allowed to cool to 45 ^{0}C. One ml of each serially diluted solution was placed on different Petri dishes using the pour plate method. Molten yeast extract agar was aseptically poured, swirled and, then allowed to solidify. Pure culture of the organism was achieved by re-streaking on the agar plates and labelled. The properly labelled plates were later incubated for 48 h at 30 ^{0}C. The isolates were sub-cultured for organism preservation and maintenance. The *S. cerevisiae* was identified through the standard morphological, physiological and, identification methods described by Ukwuru and Awah (2013).

2.4. Experimental Diet Formulation

The experimental diets were formulated to contain 35 % crude protein. The ingredients listed in Table 1 (25 kg) were adequately mixed, and divided into five equal parts of 5 kg each per treatment. The control (T1) and each treatment (T2, T3, T4 and T5) received different inclusion levels of *S. cerevisiae* at 0, 20, 25, 30 and 35 ml/kg, respectively. Each treatment was thoroughly mixed with one litre of distilled water and the resultant mash was run into 2 mm dice of the pelleting machine to produce the same size strands of sinking pellets. The pellets were air dried at room temperature and packed in the air-tight containers, labelled and kept in the refrigerator until needed for the study. A representation of each meal was analyzed for the proximate composition (Table 2).

Table 1: Gross composition of the experimental diets for the control and the four inclusion levels

Ingredients	Percent
Maize	35.0
Fish meal	20.0
Soybean meal	20.6
Groundnut cake	20.0
Vegetable oil	2.0
Premix	1.0
Di-calcium	0.5
Phosphate (DCP)	-
Salt	0.5
Lysine	0.2
Methionine	0.2
Total	100

2.5. Growth Performance and Nutrient Utilization Indices

After the 7-day acclimatization of the experimental fish, the experimental animals were randomly placed into separate experimental tanks at the stocking rate of 10 fish per tank. Initial average weights of the experimental fish were determined and noted. The change in weights of the experimental fish was monitored fortnightly while, other growth parameters such as mean weight difference, specific growth rate, survival rate, the quantity of feed consumed, and feed conversion ratio were determined at the end of the study. The mean weight difference (MWD) of the experimental fish was determined as (Adewumi and Olaleye, 2011):

Mean weight difference (*MWD*), g
= Final weight –Initial weight

Daily feed intake (*DFI*)
= Total feed intake/rearing period (days)

The specific growth rate (SGR) of the experimental fish was determined according to Aderolu et al. (2011), which is

$$SGR = \frac{(\ln final\ weight - \ln initial\ weight) * 100}{Duration\ of\ feeding\ (days)}$$

The protein efficiency ratio (*PER*) was determined according to Owodeine et al. (2011), which is:

PER = Mean weight gain (g)/Protein intake

The protein intake percentage (*PI*)
= feed intake * %protein in the diet/100

The nitrogen metabolism was determined according to Nwanna (2003):

$$Nitrogen\ metabolism = \{0.549 * (a + b)\} * {}^{h}/_{2}$$

where: *a* = Initial mean weight of fish (g),
b = Final mean weight of fish (g) and
h = Experimental periods (Days)

Feed conversion rate (FCR) is the rate at which fish can convert the consumed fish to the flesh. This was determined (Li and Robinson, 2015) using:

FCR = Feed intake (g)/Increase in biomass

$$Biomass = fish\ weight\ (g) * No.of\ fish\ stocked$$

The survival rate (SR %) of the experimental fish-fed diets containing *S. cerevisiae* was determined by considering the difference between the initial and final population of fish stocked.

SR = 100*(Initial number of fish stocked – Mortality)/Initial number of fish stocked

Table 2: Proximate composition of the experimental diets

Parameters	T1	T2	T3	T4	T5
Crude protein (%)	35.05±0.01	34.85±0.02	34.95±0.02	34.50±0.05	34.55±0.05
Crude fibre (%)	3.60±0.00	3.30±0.22	3.10±0.51	3.50±0.05	3.30±0.28
Ash content (%)	12.80±0.04	11.50±0.33	11.50±0.53	11.60±0.33	11.80±0.61
Ether extract (%)	7.10±0.20	7.30±0.05	7.10±0.25	7.20±0.10	7.30±0.07
Moisture content (%)	5.81±0.53	6.11±0.28	5.70±0.68	6.22±0.11	6.30±0.02

2.6. Challenge-test Experiment

This test was carried out immediately after the growth study by randomly selecting fifteen fish from each treatment. The fish were stocked at the rate of five fish per tank and replicated thrice. The pure culture of *Aeromonas hydrophila* was obtained from the laboratory stock of the Microbiology Department of University of Ibadan, Nigeria. The challenge solution was prepared according to Akanmu *et al*. (2022). The fish were challenged with *A. hydrophila* by injecting one ml of the challenge solution into each experimental fish intramuscularly. One ml of the challenge solution was found to contain $0.923x10^6$cfu/ml of *A. hydrophila* through the plate count method. All the challenged fish were kept under observation for 14 days and fed 3% body weight according to their treatment groups. Records of mortality and, clinical signs were closely monitored while the value for the relative percentage survival (RPS) of the challenged fish was determined according to Omitoyin et al. (2019) at the end of the challenge test. After the challenge experiment, 30 mL of 10% formalin was added to the solution in each tank for 10 min (Dapgh et al. 2019). The solution was then autoclaved for 15 min at 121 °C before disposing into the septic tank. The containers were later washed, dried and kept for subsequent use. The ethical measures were adequately taken while conducting the study. The challenged fish and water were treated adequately before disposed and overall, the study was conducted in accordance with the care and use of laboratory animals.

2.7. Hematological Study

At the end of the challenge experiment, blood samples were collected from five experimental fish which were randomly selected from each treatment. The experimental fish were drained very close to their genital openings. Four ml of blood samples was collected from the five selected fish. The blood samples were collected into two different containers. The first portion was collected into a set of plastic vials containing ethylene-di-amine tetra-acetic acid (EDTA) for hematology analysis while the second portion was transferred into the set of plain bottles for the serum biochemical analysis. The blood sample vials were transferred with ice packs to the hematology laboratory of Veterinary Medicine, the University of Ibadan for analysis.

2.8. Data analysis Methods

The data were analyzed using SPSS software (IBM Statistics 21 Version) package. The descriptive statistics was used to determine the mean and standard error of the mean values of each parameters measured. The mean values of the proximate composition of the diets, the growth, hematological and serum biochemical parameters were compared using one - way analysis of variance. The post hoc test of this study was done by using Duncan multiple range test (DMRT) to compare the mean values for the groups in homogenous subsets and the means significant levels were determined at 0.05 probability value ($P>0.05$). All the results of this study were reported as the mean values ± standard error.

3. Results

3.1. Effects of *S. cerevisiae* on Growth Performance and Feed Utilization

Growth performance and nutrient utilization of *O. niloticus* juveniles on diets of *S. cerevisiae* at various inclusion levels are presented in Table 3. The final weight, mean weight difference, percentage weight gain, percentage survival, nitrogen metabolism values increased as the level of *S. cerevisiae* in the experimental diets increased. The control experiment was observed to have the highest feed conversion ratio (FCR), while the least FCR was recorded in T2 which did not statistically differ from T4. The highest specific growth rate (SGR) was observed in T5 while, T1 had the lowest SGR. The protein intake and, protein intake percentage ratio was observed to be highest in T5, but lowest in T2. The protein efficiency ratio (PER) was highest in T2 but was significantly lowest in T1. The *O. niloticus* juvenile fed with experimental diet T5 had the highest survival rate, while the control diet recorded the least.

Table 3: Growth performance and nutrient utilization of *O. niloticus rear on meal fortified with S. cerevisiae*

Parameters	T1	T2	T3	T4	T5
Initial weight (g)	5.37±0.12	5.67±0.44	5.23±0.32	5.20±0.12	5.13±0.20
Final weight (g)	64.40±0.06^{e}	71.90±0.37^{d}	90.57±1.46^{c}	109.23±0.52^{b}	120.23±0.64^{a}
Mean weight gain (g)	59.03±0.15^{e}	66.23±0.10^{d}	85.34±1.27^{c}	103.96±0.62^{b}	115.10±0.57^{a}
Initial biomass	161.10±1.10^{b}	170.1±2.43^{a}	156.9±1.98^{c}	158.1±1.45^{c}	153.9±2.11^{c}
Final biomass	1545.60±14.09^{e}	1869.40±21.11^{d}	2445.39±10.16^{c}	2949.21±15.21^{b}	3366.40±12.11^{a}
Change in biomass	1384.50±9.23^{a}	1699.30±6.33^{d}	2288.49±5.98^{c}	2791.11±2.22^{b}	3212.54±4.54^{a}
Total feed consumed (g)	1959.20	1798.35	2676.55	2991.21	3586.89
% Protein intake	0.56±0.05^{d}	0.52±0.05^{d}	0.77± 0.01c	0.87±.0.03^{b}	1.04±0.00^{a}
Feed conversion ratio	1.42±0.02^{a}	1.06±0.00^{c}	1.17±0.03^{b}	1.07±0.00^{c}	1.12±0.01^{b}
Specific growth rate	2.16±0.04^{e}	2.21±0.06^{d}	2.54±0.02^{c}	2.73±0.01^{b}	2.84±0.03^{a}
Protein efficiency ratio	1.06±0.00^{c}	1.28±0.02^{ab}	1.11±0.00^{c}	1.20±0.01^{b}	1.37±0.01^{a}
Nitrogen metabolism	1608.76±18.35^{e}	1788.61±10.07^{d}	2208.96±11.21^{c}	2638.53±9.45^{b}	2890.65±9.92^{a}
% Survival	80.00±0.02^{c}	88.00±0.02^{b}	90.00±0.01^{ab}	90.00±0.00^{ab}	94.00±0.02^{a}

Mean values with the same superscript on the same row are not significantly different (P>0.05); values with different superscripts in the same row differ significantly ($p < 0.05$).

The experimental diets were well utilized without any side effects on the experimental fish, as reflected in the weight gain in all the experimental fish. The significant increase in weight gain recorded in the experimental fish-fed diets containing *S. cerevisiae* is in agreement with Akanmu et al. (2022), who reported weight increase in *Heterobranchus bidorsalis* juveniles fed diets fortified with *S. cerevisiae*. This result also agrees with study by Mamun et al. (2018), in which the increase in weight gain as an indication of feed acceptability and well utilization were described. The increase in weight gain of the experimental fish-fed diets containing *S. cerevisiae* could be attributed to the presence of growth stimulants in this yeast (Lara-Flores et al., 2010). It could be observed from the study that the monitored growth parameters were better with the increase in the level of *S. cerevisiae*. The highest difference in biomass and daily weight gain values over that of the control experiment is also an indication that diet T5 was well utilized by the *O. niloticus* in the study than the other experimental diets. Thus, it could be inferred from this study that the best growth rate experienced with *O. niloticus* fed diets containing *S. cerevisiae*, at 35 ml/kg over the control diet, could be attributed to the production of digestive enzymes (amylase) which increase digestibility to enhance higher growth (Opiyo et al., 2019).

The feed conversion ratio (*FCR*) is a measure of how well feed is utilized and converted to flesh by an animal. The FCR recorded in the diets containing *S. cerevisiae* was observed to be lower than that recorded in the control diet. This result agrees with the study by Opiyo et al. (2019), who also recorded low *FCR* values in *O. niloticus* fingerlings fed with probiotic diets. The specific growth rate (*SGR*) signifies the increase in the population of the unit biomass concentration (Bhatia et al., 2015). The *SGR* was observed to be increasing as the rate of *S. cerevisiae* inclusion in the experimental diets increased. Better *SGR* recorded in the diets containing *S. cerevisiae* could be attributed to higher feed consumption and its good utilization which resulted in high weight gain. The highest survival rate recorded in the experimental fish fed with diet T5 agrees with Akanmu et al. (2022) that reported a higher survival rate in *H. bidorsalis* juvenile fed probiotic diets over those fed with the control diet. The better results observed with the use of *S. cerevisiae* as probiotics on Nile Tilapia validates that probiotics enhance nutrient utilization through efficient feed conversion, stabilizing the intestinal microbes and stimulating the digestive enzyme of the host animals (Balcazar, 2006). The higher survival rate could also be linked to conducive environment being bought about by good water quality. The probiotics had not only worked on the diets but also on the water quality as reported by Balcazar (2006), who described probiotics to be able to play effective roles in improving the water quality of the culture system.

3.2. Challenge-test Experiment

The results of the experimental fish challenged with *Aeromonas hydrophila* are presented in this sub-section. Fifteen fish were infected in all the five experimental

setups. Higher mortality (10 out of the 15) of *O. niloticus* challenged with *A. hydrophila* occurred in those fed with diets T1, T2 and, T3. However, both T4 and T5 recorded only 6 mortality out of the 15. Thus, the percentage survival of the fish fed with diets T1, T2 and, T3 were 33.3% while, those of T4 and T5 were 60%, after being challenged with *A. hydrophila*. This depicts that the microorganisms in *S. cerevisiae* included in the experimental diet below 30 ml/kg did not enhance the defensive mechanism of *O. niloticus* against *A. hydrophila* as in the diets containing *S. cerevisiae* above 30 ml/kg. The fish were observed to show some clinical signs which were similar to findings in Akanmu et al., (2022), where general weakness, low response to feeding, hemorrhage and mortality were displayed by H. bidorsalis juveniles fed fortified diets and challenged with A. hydrophila.

Although, Abdel-Taawab et al. (2008) reported beta-glucans, nucleic acid and, mannan oligosaccharides present in *S. cerevisiae* as the immune-stimulating compounds that enhance the immune response in fish, the results of the challenge study showed that lower inclusion level of *S. cerevisiae* might not be able to produce the immune response required to defend *O. niloticus* from the pathogenicity of *A. hydrophila* as recorded in the diets containing *S. cerevisiae* above 30ml/kg. The better results recorded in the blood parameters of the Nile Tilapia fed treated diets over the control diet agreed with Balcazar (2006) who identified the propensity of probiotics to colonize the gastrointestinal tracts (GIT) of the host animal as a factor required to improve the mucosal secretion by producing the immune molecules in the host. The higher inclusion rates of *S. cerevisiae* could have allowed for more colonization leading to the production of more immune molecules in the fish that consumed diets containing above 30 ml/kg. However, the highest relative percentage survival (RPS) recorded in diets T4 and T5 could be a reflection of *S. cerevisiae* being able to colonize and compete for nutrients and space, thus able to stimulate defense mechanisms in *O. niloticus* juveniles at higher inclusion rate (Dawood *et al*., 2018).

3.3. Effects of *S. cerevisiae* on Hematological Parameters

Table 4 presents the results of the hematological parameters of *O. niloticus* juveniles monitored after they have been fed with diets containing *S. cerevisiae* and challenged with *A. hydrophila* as a pathogenic organism. The white and red blood cells, hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), platelets and monocytes were not significantly different among the treatments. Although these parameters did not show a statistically significant difference among the treatments, the values slightly increased with the increase in inclusion level of *S. cerevisiae* to the diets, with the highest values being observed in T5. The remaining hematological parameters; namely Packed cell volume (PCV), MCV (Mean corpuscular volume), MCH (Mean corpuscular hemoglobin), lymphocyte and neutrophils, monitored in this study showed progressive increase with the inclusion level of the *S. cerevisiae* rise. Thus, the highest values of all these parameters were obtained in the fish fed with diet T5, while the least values belong to in restricted diet (T1). The trend of the hematological parameters recorded in this study are similar to that if Soroush et al. (2011) who experimented with Common Carp (*Cyprinus carpio*) and *H. bidorsalis* juvenile fed probiotic diets.

Critical analysis of blood parameters could give a vivid information about the welfare of a fish (Esmaeili, 2021). He clearly stated that blood could determine the nature of stress and health of a fish. The supply of oxygen and nutrient to the cell tissues, waste removal, immunological function, coagulation and messenger functions have been identified to be the major blood's functions (Seibel et al, 2021). Jan et al. (2021) compared the results of blood parameters between treatments of the same experimental design as a better way of analysing the parameters. Various factors such as environmental factor, nutritional factor, and handling process during sampling among others have been reported to contribute to variations experienced in the blood parameters of fish of the same species (Esmaeili, 2021). Hence, comparing the bold parameters of the experimental fish within the design showed that Nile Tilapia fed diet containing *S. cerevisiae* at 35 ml/kg had the best health status.

Table 4: Hematological parameters of *O. niloticus* juveniles monitored after being fed with diets containing *S. cerevisiae* and challenged with *A. hydrophila*

Parameters	T1	T2	T3	T4	T5
PCV (%)	13.00±1.11^{c}	13.00±1.05^{c}	14.00±0.31^{b}	15.00±0.02^{b}	17.00±0.05^{a}
Hb (g/dl)	4.20±0.02	4.20±0.29	4.50±0.05	4.60±0.11	4.60±0.87
RBC (10^4/mm^3)	3.10±0.15	3.12±0.08	3.13±0.10	3.14±0.09	3.15±0.07
WBC (10^4/mm^3)	5.20±0.22	5.40±0.09	5.60±0.03	5.80±0.05	5.90±0.03
Platelets (10^4/mm^3)	3.00±0.01	3.00±0.01	3.00±0.00	3.00±0.01	3.00±0.00
MCV (FL)	41.00±1.67^{c}	44.00±0.12^{b}	44.00±0.07^{b}	45.00±0.03^{b}	47.00±0.01^{a}
MCH (pg)	13.00±0.87^{c}	14.00±0.62^{c}	14.00±0.58^{c}	16.00±0.22^{b}	18.00±0.13^{a}
MCHC (g/dl)	32.00±0.08	32.00±0.03	32.00±0.03	32.00±0.05	32.00±0.03
Lymphocyte (%)	53.00±2.31^{d}	56.00±1.07^{c}	56.00±1.23^{c}	58.00±0.68^{b}	60.00±0.09^{a}
Neutrophils (%)	41.00±0.67^{d}	43.00±1.27^{c}	44.00±1.58^{c}	46.00±0.42^{b}	48.00±0.39^{a}
Monocytes (%)	1.00±0.01	1.00±0.00	1.00±0.01	1.00±0.00	1.00±0.00

PCV: Packed cell volume; Hb: Hemoglobin; RBC: Red blood cell count; WBC: White blood cell count; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration. Mean values with the same superscript on the same row are not significantly different (P>0.05); values with different superscripts in the same row differ significantly ($p < 0.05$).

The Hemoglobin values in fish indicate the amount of oxygen in the blood to be transported to the cell tissues. Therefore, the high levels of hemoglobin recorded in the diets T4 and T5 could signify high delivery of oxygenated blood in the experimental fish fed with diets T4 and T5, probably making them to be healthier and stronger than those fed diets containing *S. cerevisiae* at inclusion levels below 30 ml/kg of fish feed. Esmaeili (2021) described hemoglobin and red blood cell counts to play a vital role in the fish metabolism and fish growth. This could be the reason for the better growth rate and well nutrient utilisation by *O. niloticus* fed diet T5.

Table 5 depicts the serum biochemical results of *O. niloticus* juveniles monitored after been fed with diets containing *S. cerevisiae* and trial with *A. hydrophila*. The values of all the parameters monitored were also observed to be on the increase as the inclusion level of the *S. cerevisiae* increased in the experimental diets. The values for aspartate transaminase (AST), total serum protein (TP), albumin (ALB), globulin (GLB), alanine transaminase (ALT), and creatinine (CRT) were highest in T5, while the least values of these parameters were in T1.

The results of the serum biochemical examination of blood constituents of the experimental fish reflected the two enzymes monitored (Aspartate transaminase and Alanine transaminase) showed significant differences within the treatments while other parameters were not significantly different within the treatments. However, the highest values were also observed in T5 and this verifies the inclusion of *S. cerevisiae* at higher inclusion levels. The total serum protein has been linked to fish humoral immunity and innate immune response (Osman et al., 2019). Therefore, the higher survival rate recorded in *O. niloticus* fed diet T5 could be attributed to higher value for total serum protein recorded.

Table 5: Serum biochemical results of the blood constituents of *O. niloticus* juveniles monitored being fed with diets containing *S. cerevisiae* and challenged with *A. hydrophila*

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
Total Serum Protein	3.10±0.05	3.12±0.02	3.14±0.04	3.14±0.03	3.20±0.07
Albumin	1.00±0.02	1.02±0.01	1.10±0.03	1.10±0.01	1.10±0.01
Globulin	2.03±0.12	2.04±0.09	2.10±0.06	2.10±0.08	2.12±0.04
Creatinine	1.00±0.01	1.01±0.01	1.02±0.00	1.05±0.01	1.10±0.00
Aspartate transaminase	30.00±3.55^{c}	30.00±2.65^{c}	32.00±1.87^{b}	32.00±2.89^{b}	34.00±2.34^{a}
Alanine transaminase	18.00±2.26^{e}	20.00±1.00^{d}	22.00±0.06^{c}	22.00±0.04^{b}	24.00±0.04^{a}

Mean values with the same superscript on the same row are not significantly different (P>0.05); values with different superscripts in the same row differ significantly ($p < 0.05$).

4. Conclusions and Recommendations

The use of *Saccharomyces cerevisiae*, a fungi as probiotics in the conventional feed of Nile Tilapia was studied to enhance the growth rate and survival rate and to improve the blood constituents of *O. niloticus* juveniles. It will therefore be very imperative to consider the use of probiotics, especially *S. cerevisiae*, in the fish feed production of Nile Tilapia and other fish species in order to produce healthy fish at fast growing rate, and thus make the aquaculture business a lucrative one. Further studies, to determine the best inclusion rate above 35 ml/kg of *S. cerevisiae* on Nile Tilapia and other fish species, is recommended to be conducted to establish if inclusion level above 35 ml/kg will give better results on Nile Tilapia and possibly on other fish species. Efforts should also be made into mass production of this probiotics at its best inclusion level and made readily available at reasonable and affordable cost for the fish farmers who have the desire to prepare their fish feeds by themselves. For the fish farmers who may not want to be involved in the feed production, the finished fish feed products containing *S. cerevisiae* at its best inclusion level should also be made readily available at an affordable rate.

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Reference

Abdel-Tawwab, M., Abdel-Rahman, A. M. and Ismael, N. E. M. (2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile Tilapia, *Oreochromis niloticus* (L) challenged in situ with *Aeromonas hydrophila*. *Aquac.,* 280(1-4): 185-189.

Aderolu, A. Z., Aarode, O. O., Akonu, A.A. and Jimoh, W. A. (2011). The effect of substituting maize with graded level of biscuit waste on growth, nutrient utilization, carcass composition, hematological parameter and economic performance of African catfish (*Clarias gariepinus*). *J. Nat. Sci., Eng. Technol.,* 10(2): 111-120.

Adewumi, A. A., and Olaleye, V. F. (2011). Catfish culture in Nigeria: Progress, prospects, and problems. *Afr. J. Agric. Res.,* 6(6): 1281-1285.

Akanmu, O. A. (2018). Probiotics, an alternative measure to chemotherapy in fish production (Chapter 9). In: 'Probiotics–current knowledge and future prospects, Ed. Enany, S. Intech Open, London, United Kingdom. pp. 151-168.

Akanmu, O.A., Omitoyin, B. O., Kolawole, A. E. and Emikpe, B. O. (2022). Effect of dietary *Lactobacillus fermentum*, and *Saccharomyces cerevisiae* on the growth performance, hematological parameters, organ-somatic indices, and protection of *Heterobranchus bidorsalis* juveniles against *Aeromonas hydrophila* infection. *J. Appl. Aquac.*, 34(2):358-381.

Balcazar, J. L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Muzquiz, J. L. (2006). The Role of Probiotics in Aquaculture. *Vet. Microbiol.,* 114(3-4):173-186.

Bene, C., Arthur, R., Norbury, H., Allison, E. H., Beveridge, M., Bush, S., Campling, L., Leschen, W., Little, D., Squires, D., Thilsted, S. H., Troell, M. and Williams, M. (2016). Contribution of Fisheries and Aquaculture to Food Security and Poverty Reduction: Assessing the Current Evidence. *World Dev.,* 79:177-196.

Bhatia, S., Sharma, K. and Bera, T. (2015). Plant tissue culture. In: Modern Application of Plant Biotechnology in Pharmaceutical Sciences. Elsevier Inc., pp. 31-107.

Dapgh, A. N., Hakim, A. S., Shawky, H. and Ibrahim, E. S. (2019). Study of some disinfectants efficacy on *Aeromonas hydrophila* recovered from local animal and water sources. *J. Agric. Vet. Sci*, 12 (2):41–47.

Dawood, M. A. O., Koshio, S. and Esteban, M. A. (2018). Beneficial roles of feed additives immunostimulants in aquaculture: A review. *Rev. Aquac.,* 10(4):950–974.

Esmaeili, N. (2021). Blood performance: A New Formula for Fish Growth and Health. *Biology*, 10(12):1236.

FAO (2018). Culture aquatic species information program: *Oreochromis niloticus* (Linnaeus 1758). FAO Fisheries and Aquaculture Department.

FAO (2022). The State of World Fisheries and Aquaculture 2022 - Towards Blue Transformation. Food and Agricultural Organization of the United Nations, Rome, Italy.

FAO (2023). Top 10 species groups in global, region and national aquaculture 2021. A Supplementary materials to the factsheet on Top 10 species groups in global aquaculture 2021. Food and Agricultural Organization of the United Nations, Rome, Italy.

Fashina-Bombata, H. and Megbowon, I. (2012). Proximate composition and breeding description of an unidentified Cichlid of Epe Lagoon, Lagos, Southwest Nigeria, commonly called 'Wesafu'. *Int. J. Nut. Metab.,* 4(4):57-63.

Ghodratizadeh, S., Ghodratizadeh, S., Farhoudi, M. and Habibian, R. (2011). Effects of addition of *Saccharomyces cerevisiae* and *Bacillus subtilis* in diet on selected haematological and biochemical parameters in common carp (*Cyprinus carpio*) *World J. Fish & Marine Sci.,* 3(1): 96 – 99.

Jan, K., Ahmed, I. and Dar, N. A. (2021). Haematological and serum biochemical reference values of snow trout, *Schizothorax labiatus* habiting in river Sindh of Indian Himalayan region. *J. Fish Biol.,* 2021, 98(5):1289–1302.

Jenkins, J. A., Bart, H. L., Bowker, J. D., Bowser, P. R., MacMillan, J. R., Nickum, J. G., Rachlin, J. W., Rose, J. D., Sorensen, P. W., Warkentine, B. E. and Whitledge, G. W. (2014). Guidelines for Use of Fishes in Research-Revised and Expanded. *Fisheries*, 39(9): 389-436.

Lara-Flores, M., Olivera-Castillo, L. and Olvera-Novoa, M. A. (2010). Effect of inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus*) and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization, and intestinal enzymatic activity of Nile Tilapia (*Oreochromis niloticus*). *Int. J. Fish. Aquac.,* 2(4):93–101.

Li, E.H. and Robinson, M. H. (2015). Complete feeds-intensive systems. In: Feed and feeding practices in Aquaculture, Ed. Davis, D. A. Wood-head Publishing Series in Food Science, Technology and Nutrition, pp. 111-126.

Mamun, M.A., Nasren, S. and Bari, M. S. (2018). Role of probiotics in aquaculture: Importance and future guidelines. *J. Bangladesh Acad. Sci.,* 42(1):105–09.

Nwanna, L. C. (2003). Nutritional value and digestibility of shrimp head waste meal by African catfish, *Clarias gariepinus*. *Pak. J. Nut.,* 2(6):339-345.

Oluwalola, O. I., Fagbenro, O. A. and Adebayo, O. T. (2020). Comparative Study on Growth and Economic Performances of Nile Tilapia, *Oreochromis niloticus* Reared Under Different Culture Enclosures in Akure, Nigeria. *Aquac. Stud.,* 20(2):91-98.

Omitoyin, B.O., Ajani, E.K., Orisasona, O., Bassey, H.E., Kareem, O.K. and Osho, F. E. (2019). Effect of guava *Psidium guajava* (L.) aqueous extract diet on growth performance, intestinal morphology, immune response, and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Aquac. Res.,* 50(7):1851–61.

Opiyo, M. A., Jumbe, J., Ngugi, C. C. and Charo-Karisa, H. (2019). Different levels of probiotics affect growth, survival, and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Sci. Afr.,* 4: e00103.

Osman, A., Hamed, M. and Sayed, A. (2019). Protective role of *Spirulina platensis* against UVA-induced haemato-biochemical and cellular alterations in *Clarias gariepinus*. *J. Photochem. Photobiol B.* 191:59–64.

Owodeinde, F. G, Ndimele P. E., and Anetekhai, M. A. (2011). Reproductive, growth performance and nutrient utilization of *Heterobranchus bidorsalis* (Geoffroy, 1809) and its Hybrid "Clariabranchus" induced with synthetic hormone and pituitary gland of *Heterobranchus bidorsalis*. *Int. J. Zool. Res.,* 7(5): 345-357.

Samaddar, A. (2022). Recent Trends on Tilapia Cultivation and its Major Socioeconomic Impact among Some Developing Nations: A Review. *Asian J. Fish. Aquat. Res.,* 16(4): 1-11.

Seibel, H., Babmann, B and Rebl, A. (2021). Blood will tell: what hematological analyses can reveal about fish welfare. *Front. Vet. Sci.,* 8: 616955.

Sharma, S., Dahiya, T., Jangra, M. Muwal, A. and Parihar, C. S. (2022. *Saccharomyces cerevisiae* as probiotics in aquaculture. *J. Entomol. Zool. Stud.,* 10(1):101-104

Ukwuru, M. U. and Awah, J. I. (2013). Properties of palm wine yeasts and its performance in wine making. *Afr. J. Biotechnol.,* 12 (19):2670-2677.