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The effect of water aquaria change on nutrient utilization and microbial activity of Nile tilapia *Oreochromis niloticus*

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Abstract
Nutrient utilization was evaluated in Nile tilapia (*Oreochromis niloticus*) where it was divided into four groups according to time of water replacing (after 2, 4, 6 and 8 days for control, T1, T2 and T3, respectively). Final protein efficiency ratio values of T1 were significantly higher than other groups (P<0.05). The best (lowest) mean feed conversion ratio (FCR) was obtained from T1, while it was in T1 non-significantly different (P>0.05) with control. The feed intake and protein intake showed similar values in all groups. The best Nutrient utilization parameters were in T1, which decreased the cost of fish production by decrease in the fuel consumption. The maximum value of total bacterial count was in the fish intestine in T3 being 1.96×10^6 Colony Forming Unit (CFU) /g after 12 weeks. Yeast and fungal count in the fish intestine fluctuated between 0.05 and 12.2 $\times10^2$ CFU/g. Coliform count in the fish intestine up to the minimum values in the first week being 0.34, 0.23, 0.31 and 0.3 $\times10^2$ CFU/g in C1, T1, T2 and T3, respectively. Five different colonies of fungal isolates were identified as *Aspergillus ochraceus*, *A. oryzae*, *A. niger*, *Geotrichum candidum* and *Penicillium* sp.

Keywords: Protein use, micro-organisms, fish feed, Nile Tilapia

1. Introduction
Fish is considered the primary source of protein in many parts of the world, rich in essential amino acids, micro elements such as fluorine, iodine etc and macro elements such as calcium, phosphorus etc, fats that are valuable sources of energy, fat-soluble vitamins and unsaturated fatty acids, among other benefits, have a hypocholesterolemic effect (anti-arteriosclerosis) [1]. Aquaculture is the world's fastest growing agro-food sector, average annual growth rate of 8.8% over the last 30 years. The global annual production is expected to grow from 72.1 million metric tons in 2006 to approximately 230 million metric tons by 2030 to meet the human demands for protein. Fish culture is a good source of income and employment for hundreds of millions of people around the world. Aquaculture is the fastest-growing animal-food-producing sector, now accounting for almost half of total food fish supply. Among the species selected to portray yield potential in Africa for aquaculture production are Nile tilapia (*O. niloticus*) [2]. Tilapias are considered as the best species for culture because of their high tolerance to both adverse environmental conditions and relatively poor water quality, their relatively fast growth, disease resistance, ability to grow and reproduce in captivity, and feed on low trophic levels. Therefore, they have become an excellent choice for aquaculture, especially in tropical and subtropical environments [3-5]. Climate changes affecting freshwater resources and causing decline in water in the world have been associated with temperature and rainfall fluctuations [6]. Fish production requires water for providing oxygen and clean metabolites waste [7]. The water exchange rate was clearly affected in feed conversion ratio and the growth rate of shrimp [8, 9]. Feed conversion ratio (FCR) is an important parameter for the determination of the amount of feed consumed. The feed conversion ratio of a fish is a remarkable tool to compute the acceptability of artificial feed [10, 11].
Nutrition is one of the most important factors influencing the ability of cultured fish to exhibit its genetic potential for growth and reproduction. They are also greatly influenced by factors such as behaviour of fish, quality of feed, daily ratio size, feed intake or water temperature [12]. The Fish feed generally constitutes least 60% of the operational cost in the intensive and semi-intensive aquaculture system.

The need to minimize feed cost through the use of newer and cheaper sources of feed ingredients, has already been considered. Selection of feed ingredients for use as a fish feed will play a major role in matching its ultimate nutrition in addition to economic success. The feed utilization efficiency of *O. niloticus* affected by different environmental factors such as pH, nitrogen waste, temperature, dissolved oxygen concentration and other water quality parameters. Culture conditions, including food quality, amount of feed provided, and water temperature affect the optimum feeding frequency for maximum growth of fish [5, 13].

The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in color, odor and texture. Spoilage of fresh and lightly preserved fish products is caused by microbial action. Microbiological spoilage of foods may take diverse forms, but all of them are a consequence of microbial growth and/or activity, which manifests itself as changes in the sensory characteristics [14]. Some of microorganisms that live in aquatic environments and associate with fish may cause disease. Biological pollutants may also introduce pathogens into the aquatic environment that could lead to the death of aquatic food organisms such as fish and snails. These pathogens may be transferred to human beings through fish consumption. Fresh water fish may harbor human pathogens after exposure to contaminated water or food sources. Fish samples were seen to harbor of *Aeromonas hydrophila, Escherichia coli*, *Salmonella* species and *Shigella* species [15, 16].

The most common species found in the gastrointestinal tract of Nile tilapia belong to Gram-negative bacterial species: *A. hydrophila*, *A. veronii*, *Burkholderia cepacia*, *Chromobacterium violaceum*, *Citrobacter freundii*, *E. coli*, *Flavimonas oryzihabitans*, *and Plesiomonas shigelloides* [17, 18]. However, the presence of potential human pathogens suggests that fish improperly handled, undercooked or consumed raw may cause various diseases in susceptible individuals [19, 20].

The establishment of a balanced gut microflora is important in animal health and digestive function and it can possibly occur in fish, where a resident microflora has been suggested. The microbiology of the fish gastrointestinal tract has been subjected to many researches. Characteristics of the microenvironments at various locations throughout the alimentary tract influence both the taxonomic composition and the numerical abundance of bacteria present and other microorganisms. The gastrointestinal tract of fish can influence nutrition, growth, and disease susceptibility. The microflora may be essential in fish that feed on material lacking vitamins, which the microflora can synthesise [18, 21].

Bacterial populations of fish skin ranged from 10^2 to10^4 (CFU/cm^2). Gill tissue has been found to harbor high bacterial populations, e.g., up to 10^6 CFU/g. Muscle has been considered by some authors to be sterile [22]. Bacterial populations in the digestive tract can be up to 10^8 for aerobic heterotrophs and 10^5 for anaerobic bacteria CFU/g. Fish eggs may be populated by high numbers of bacteria 10^3–10^6 CFU /g. Incidentally, the digestive tract of newly hatched larvae contains scant bacterial populations, but are quickly colonized[23]. Fish of a good quality should have counts of total bacteria less than 10/g. faecal coliforms and total coliforms should not exceed 10/g and 100/g, respectively. Total coliform count in water is not higher than of World Health Organization standard (WHO) ($1.0x10^3$ CFU/100 ml) [15]. Therefore, the present study was performed to prove if water aquariums change will help to decrease the microbiological activity status of fish and water aquaria. Also, it investigated the nutrient utilization, survival rate of Nile tilapia reared at four different times in water replacing media to reduce the using water and replacing cost the water in aquaculture production.

2. Methods and Material

2.1 Experimental design and procedure

Tilapia fingerlings (*O. niloticus*) were obtained from Abbassa fish hatchery, General Authority for Fish Resources Development, Abbassa, Abo-Hammad, Sharkia, Egypt. Initially, fish stayed in the glass tanks for one week for acclimatization to laboratory conditions. Tilapia fingerlings were divided into Four groups with two replicates for each treatment comprising twenty individuals (weight 1.33 – 1.36 g/fish and length 4.0 – 4.1 cm/fish) in each experimental tank containing 200 litres of water. The feed was containing 35% CP and supplied at the rate of 3% of wet body weight of fish fingerlings twice a day for a period of 12 weeks (90 days). Diet formulations are presented in (Table 1). Fish tanks were cleaned and collected the faeces before water replacing once in every group. Fish tanks were cleaned and collected the faeces before water replacing in every once according to group. Each tank contains compressed air to supply oxygen into the experimental tanks. The water supply was provided from a storage fiberglass tank where, the water was replaced with rate of 50% per once according to the group. First group (control) was replacing water every two days and second group was replaced water every four days while, a third group was replacing the water every six days. Finally, the fourth group was replaced water every eight days. Growth was estimated by weighing the fish in each tank. Total lengths and body depth of the fish were measured every two weeks.

The amount of the feed was adjusted once in two weeks intervals based on the body weight of the fish. Thus, the amount of daily feed ration (DFR) at each sampling time was calculated using the mean body weight (MBW) and the total number of the fish (N) and the feeding rate per day (FR/d) using the following formula: DFR= (MBW×N×FR)/d [24].

2.2 Proximate analysis of diet and fish

The tested diets and fish from each treatment were chemically analyzed according to the standard methods of AOAC [25] for moisture, protein, fat and ash. The moisture content was estimated by heating samples in an oven at 85 °C till constant weight and calculating weight loss were obtained. Nitrogen content was measured using a micro kjeldahl apparatus and crude protein was estimated by multiplying nitrogen content by 6.25. Total lipids content was determined by ether extraction for 16 hr and ash was determined by combusting samples in a muffle furnace at 550 °C for 6 hr. Crude fibre was estimated according to [26].

2.3 Data analysis

From the experimental data obtained in replicate tanks feed conversion ratio (FCR) and Protein efficiency ratio (PER) were calculated as described by [27].

Weight gain = W2 – W1

FCR = Food Fed/Live Weight Gain

Protein efficiency ratio (PER) = live weight gain (g) / protein fed (g)

Protein intake = (CP% * feed intake) /100

Survival (%) = F2/F1× 100

Where; W1 = initial weight of fish, W2 = final weight of fish,

F1 = number of fish at the end of the experiment, F2 = number of fish at the beginning of the experiment.

2.4 Statistical analysis
PROC GLM procedure of the Statistical Analysis Systems[28] was used to analyze the Least-squares means (LSM) and standard errors (SE) in each level of Treatments and the differences between means were detected by Duncan`s Multiple Range Test [29]. Regression coefficient analyzed with original data.

2.5 Microbiological examinations
The tested samples (fish and water) were collected from animal production laboratory and transferred to the microbiology laboratory into the icebox. One ml of samples obtained from each water tank or one gram of each fish intestine sample was aseptically transferred to 9 ml of sterile water. For the microbiological examination of fish surface, 10 ml of sterile water were aseptically transferred to a plastic bag containing the tested fish and samples were shaken manually for 2 min, the suspension was collected aseptically in sterilized test tube. The suspension of all samples were shaken for 10 min using a vortex (VM-300 power: 220 VAC, 50Hz, 0.16A/Made in Taiwan-Associated with Cannic, in the U.S.A.) to homogenate the obtained solution. Serial dilutions were performed and one ml of each last three dilutions was used for microbiological examinations [14].

2.6 Total bacterial count
Poured plate method was used, after preparing suitable serial dilutions of fish intestine samples, 1 ml was transferred into sterile glass Petri dishes in triplicates. Approximately 15 ml of melted nutrient agar medium (45-50 °C) was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 30 °C for 72 hours in a digital incubator (Switc, MPM Instruments s.r.l., Bernareggio/Made in Italy). After the incubation period, developed colonies were counted per each plate of the same dilution. The total colonies count per gram or ml of samples was calculated as follows: Total bacterial count = average number of triplicate plates of the same dilution x reciprocal of the dilution used (CFU) /ml or g sample [14].

2.7 Total yeast and fungal count, isolation and Maintenance
One ml of suitable folds serial dilutions of all fish intestine samples were inoculated onto three plates using poured plate method. Approximately fifteen ml of potato dextrose agar (PDA) medium (PDA) at about 50 °C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 25 °C for 5 days in the incubator. After the incubation period, developed colonies were counted per each plate. The mean count of plates was recorded to represent fungal count. Single different developed colonies were isolated on a PDA medium slant for identification tests. The fungal isolates were subcultured then maintained on PDA slants at 5 °C till use [31].

2.8 Count of coliform group
Coliform counts were estimated based on the most probable number (MPN) technique. Three decimal dilutions for each sample in three replicate tubes were employed. One ml of each suitable dilution was added to test tubes containing MacConkey broth medium and Durham tubes, then incubated at 37ºC for 48 hours in the incubator. The number of positive tubes showing acid and gas were recorded. The MPN of coliform bacteria per gram of sample was calculated from standard Table according to [32].

2.9 Identification of fungal isolates
Potato dextrose agar (PDA) medium [33]. Rose Bengal yeast extract sucrose agar (RYS) medium [34]. was used for identification of isolated fungi. Fungal isolates were identified by morphological characteristics of colonies in different cultivation media such as PDA and RYS. In addition, the vegetative and reproductive features observed using a light microscope (Olympus CX31 Binocular Halogen Microscope made in Japan) with a magnification power 400x, were also considered. The following taxonomic keys were used [35-37].

3. Results and Discussion
3.1 Chemical evaluation of fish samples
Data in Table 1 show that the chemical analyses of experimental diet fed to Nile tilapia (*O. niloticus*). Dry matter, ash, organic matter, crude protein, crude fat, fiber and nitrogen free extract were 90.96, 9.65, 81.31, 35.4, 4.35, 6.0 and 35.56%, respectively. Table 2 shows the mean body weight in all different groups at the end of treatment. Mean initial body weight was non-significantly different with each all groups. During the second and fourth weeks, mean body weight of fish in all groups was non-significantly different.

Table 1: Chemical analyses (%) of the experimental diet fed to Nile tilapia (*Oreochromis niloticus*)

Composition parameters	% DM basis based on dry matter
Dry matter	90.96
Ash	9.65
Organic matter	81.31
Crude protein	35.4
Crude fat	4.35
Fiber	6.0
Nitrogen free extract	35.56

However, during the sixth week, mean body weight of fish was significantly highest (P<0.05) for T3 and non-significantly higher (P<0.05) for T1 and T2 compared to control, but it was in T3 significantly higher than T1and T2 (P<0.05). During eighth and tenth weeks, mean of fish body weight was non-significantly different with each groups. During twelfth week, the final body weight of fish was significantly highest (P<0.05) for T1 and significantly lowest (P<0.05) for T2 and T3 compared to control. It was in T1significantly higher than T2 and T3 (P<0.05).

Table 3 shows the mean feed intake in all different groups at the end of treatment. Mean feed intake was non-significantly different with each all groups during second, fourth, sixth, tenth and twelfth weeks. During eighth week, Mean feed intake was significantly higher (P<0.05) for T3 and non-significantly different for T1and T2 compared to control. It was in T3 significantly higher than T1and T2 (P<0.05).

Table 4 shows the mean of feed conversion ratio (FCR) in all different groups at the end of treatment. During second and sixth weeks, the mean of FCR was non-significantly different between all groups. During the fourth week, mean FCR was significantly lowest (P<0.05) for T1, T2 and T3 compared to control. It was non-significantly different between T1, T2 and T3. During eighth week, mean FCR of fish was significantly highest (P<0.05) for T3 and non-significantly higher (P<0.05) for T1 and T2 compared to control. But, it was in T3 significantly higher than T1 and T2 (P<0.05). During tenth

week, mean FCR of fish was significantly highest (P<0.05) for T2 and T3, and significantly lowest (P<0.05) to T1 compared to control. It was in T1 significantly lower than T2 and T3 (P<0.05). During twelfth week, mean FCR of fish was significantly highest (P<0.05) for T2 and T3, non-significantly lower (P<0.05) for T1 compared to control. It was in T1 significantly lower than T2 and T3 (P<0.05).

Table 5 shows the mean protein intake in all different groups at the end of treatment. Mean protein intake was non-significantly different with each of the groups during second, fourth, sixth, tenth and twelfth weeks. During eighth week, Mean protein intake was significantly higher (P<0.05) for T3 and non-significantly different for T1and T2 compared to control. It was in T3 significantly higher than T1and T2 (P<0.05). *Table 6* shows the mean protein efficiency ratio of fish in all different groups at end of treatment. During second week, mean protein efficiency ratio was non-significantly different for T1 and significantly lowest (P<0.05) for T2 and T3 compared to control. It was in T1 significantly higher than T2 and T3. During fourth week, mean protein efficiency ratio of fish was significantly highest (P<0.05) for T1, T2 and T3 compared to control. It was in T2 significantly higher than T1 and T3. During sixth week, mean protein efficiency ratio of fish was significantly highest (P<0.05) for T3 and it was non-significantly different with T1 and T2 compared to control. It was in T3 significantly higher than T1and T2 (P<0.05).

During eighth week, mean protein efficiency ratio of fish was significantly lowest (P<0.05) for T1, T2 and T3 compared to control. Also, it was non-significantly different with T2 and T3 compared to T1. During tenth and twelfth weeks, mean protein efficiency ratio of fish was significantly highest (P<0.05) for T1 and significantly lowest (P<0.05) for T2 and T3 compared to control. It was in T1 significantly higher than T2 and T3 (P<0.05). Table 7 shows initial and final composition in the body of Nile tilapia fingerlings (O. *niloticus*) fed experimental diet. The body initial composition was lower than final composition in each all groups. Ash was non-significantly highest (P<0.05) forT1, non-significantly lowest (P<0.05) for T2, and it was non-significantly different for T3 compared to control in final composition. Moisture, crud protein (CP), Total lipids, nitrogen free extract (NFE) and organic matter (OM) were not affected by varying of water replacing in final composition.

The experiment was aimed at investigating nutrient utilization of Nile tilapia *O. niloticus* fingerlings during water replacing in different times. The mean body weight was improved and no mortality was incurred during the time in each experimental group of this study, which confirms the suitability of the chosen nutritional composition for tilapia juvenile. The highest body weight in treatment T1was agreement with [9] who found that the effect of water exchange rate (10 or 20% twice a weekly) of shrimp caused weight highest in shrimp receiving highest level of water exchange rate and lowest in shrimp receiving lowest level of water exchange rate. The results indicate that fish can consume the feed well and indicators attributed to completely utilize from feed.

In this study the feed contained 35% CP. The optimum dietary protein level is 35% for Nile tilapia fingerlings [38]. Increase the palatability of the diet lead to increased feed intake, and increased food consumption has impact on the final body weight [39]. Low palatability was therefore a possible cause for poor growth performance. Also, poor performance may have been caused by inadequate utilization of feed or digestion of the feed. There are indications that the digestive capacity of the prey organism is important for the digestion of the feed by the fish [40]. In the present study, it was observed that Feed conversion ratio was similar for T1 and control. Feed conversion ratio (FCR) is calculated from the number of Kg of feed used to produce Kg fish. The amount of feed consumed is a crucial factor for calculating food conversion ratio (FCR). Since feed is expensive, feed conversion ratio (FCR) is an important parameter for the determination of effective use of feed [10, 11]. The feed conversion ratio helps the farmer to feed the fish to satiation, and when fish are fed exactly the quantity of feed required, they are not stressed and they provide high quality meat for human consumption [41, 42].

[8]. Reported similar results for shrimp. They showed that feed conversion ratio were clearly affected by water exchange rate. The result indicated that fish had consumed comparatively less diet but it gained high body weight, which is in agreement with values of body weight. Protein is an essential nutrient that must be included in the diet at appropriate levels to ensure adequate growth and health of fish. Protein efficiency ratio (PER) is used as an indicator of protein quantity and quality in the fish's diet and amino acid balance. So, this parameter is used to assess protein utilization turnover, where it is related to dietary protein intake and its conversion into fish and protein gain [43]. Reported that optimum growth of fish can be achieved when their protein utilization efficiency is high.

Moreover [44]. demonstrated that the rate of absorption of amino acids from the gut of casein or whey protein-based diets affect the protein synthesis within the whole body protein catabolism and oxidation of amino acids. In general, body composition of tilapia fed varying experimental diets resulted in higher crude protein and lipid compared with the initial status. The moisture content was lower and organic matter was higher in T1 than control and other groups, indicating improvement in conversion coefficient of diet.

3.2 Microbiological evaluation of fish samples

3.2.1 The total bacterial count of Nile tilapia during twelve weeks

Data in Fig.1 showed that the values of total bacterial count in water tanks, fish surface and fish gut during 12 weeks in four tanks. During the first six weeks the values of total bacterial count in the water increased slightly by increasing the time in all groups

Table 2: Biweekly variations of mean body weight (g) of Nile tilapia (*Oreochromis niloticus*) treated at different times.

TRT	W0	W2	W4	W6	W8	W10	W12
C.	1.338	2.670	3.736	5.933	10.995	20.022	28.384^{b}
T1	1.348	2.603	3.835	6.364	10.722	20.643	31.448^{a}
T2	1.361	2.450	3.949	6.545	11.527	19.125	25.547^{c}
T3	1.344	2.530	3.698	6.509	11.498	19.159	24.204^{c}
±SE	0.020	0.108	0.174	0.299	0.488	0.677	0.843

Mean values with different superscripts within a row differ significantly (P<0.05)

Table 3: Biweekly variations of feed intake (g/day) values of Nile tilapia (*Oreochromis niloticus*) treated at different times.

TRT	W2	W4	W6	W8	W10	W12
C	0.040	0.080	0.112	0.178	0.330	0.601
T1	0.040	0.078	0.115	0.191	0.322	0.619
T2	0.041	0.074	0.118	0.196	0.346	0.574
T3	0.040	0.076	0.111	0.195	0.345	0.575
±SE	0.001	0.003	0.005	0.010	0.015	0.020

Mean values with different superscripts within a row differ significantly (P<0.05)

Table 4: Biweekly variations of feed conversion ratio (FCR) values of Nile tilapia (*Oreochromis niloticus*) treated at different times.

TRT	W2	W4	W6	W8	W10	W12
C.	0.330[b]	1.498[a]	0.584[a]	0.510	0.662[b]	0.738[b]
T1	0.362[b]	1.019[b]	0.512[b]	0.598	0.557[c]	0.706[b]
T2	0.395[a]	0.860[c]	0.47 [b]	0.576	0.794[a]	1.529[a]
T3	0.387[a]	0.822[c]	0.511[b]	0.578	0.784[a]	1.745[a]
±SE	0.018	0.068	0.045	0.031	0.028	0.109

Mean values with different superscripts within a row differ significantly (P<0.05)

Table 5: Biweekly variations of protein intake values of Nile tilapia (*Oreochromis niloticus*) treated at different times.

TRT	W2	W4	W6	W8	W10	W12
C.	0.014	0.028	0.036	0.062	0.115	0.210
T1	0.014	0.027	0.040	0.067	0.113	0.217
T2	0.014	0.026	0.041	0.069	0.121	0.201
T3	0.014	0.027	0.039	0.068	0.121	0.201
±SE	0.000	0.001	0.002	0.031	0.005	0.007

Mean values with different superscripts within a row differ significantly (P<0.05)

Table 6: Biweekly variations of protein efficiency ratio values of Nile tilapia (*Oreochromis niloticus*) treated at different times.

TRT	W2	W4	W6	W8	W10	W12
C.	103.88[a]	38.41[c]	57.96[c]	82.69[a]	78.76[b]	40.76[b]
T1	101.76[a]	54.55[b]	67.88[b]	70.47[b]	92.70[a]	48.73[a]
T2	83.90[b]	62.59[a]	69.41[b]	75.53[b]	65.94[c]	30.30[c]
T3	88.57[b]	52.58[b]	89.11[a]	77.12[b]	64.58[c]	23.02[d]
±SE	4.938	2.224	3.955	2.998	3.214	2.404

Mean values with different superscripts within a row differ significantly (P<0.05)

Table 7: Initial and final composition in the body of Nile tilapia fingerlings (*Oreochromis niloticus*) fed experimental diet (%).

Composition	Initial status	Final composition			
		Control	T1	T2	T3
Moisture	88.7±2.0	84.6±0.9	82.4±1.1	84.0 ±0.9	83.8±0.7
OM	9.30±1.0	12.64±1.06	13.86±0.97	13.76 ±0.62	13.37±0.68
Ash	1.99±0.06	2.78±0.36[ab]	3.72±0.42[b]	2.28±0.44[b]	2.89±0.33[ab]
Crude protein	7.44±0.06	8.48±0.6	9.67±0.48	8.85±0.48	9.03±0.64
Crude lipids	1.36±0.03	2.94±0.41	3.11±0.63	3.52 ±0.43	3.01±0.40
Nitrogen Free Extract	0.50±0.07	1.22±0.35	1.08±0.13	1.39±0.28	1.34±0.17

All values are mean of duplicate feeding groups and values in the same row with different superscripts are significantly different (P<0.05), Nitrogen Free Extract (NFE) = 100- (protein + fat t+ fiber + ash)

(C, control: which replaced water every two days; T1: which replaced water every four days; T2: which replace the water every six days and T3: which replaced water every eight days). By the next weeks (W8, W10 and W12) total bacterial count in the water increased markedly increase until reaching the maximum values at the end of experiment after 12 weeks being 1.93×10^4, 1.82×10^4, 2.89×10^4 and 2.88×10^4 CFU/ml in C, T1, T2 and T3, respectively. Generally the values of total bacterial count in water took the lowest values in C followed by T1 and T3 while T3 took the highest values.

Similar results were obtained from [45] who studied that total bacterial count in water as potential markers of microbial contamination, and they found that, total bacterial count varied from not detected to 2.16×10^4 CFU/ml. Our results were also similar with those obtained from [17], who published the quantitative and qualitative of bacterial flora of hybrid tilapia (*O. niloticus* X *O. aureus*) and they found that, the total bacteria were ranged from $5.6x10^3$ to $24x10^3$ CFU/ml in water. Our aquarium was less in microbial pollution than [46] who study the count of total bacteria of *O. niloticus* in an aquarium experiment and they found that, the total bacteria count was $4.4x10^4$ CFU/ml in water. On the other hand, [47]. found that total bacterial count in water of puffer fish (*Fugu niphobles*) housed in glass aquaria ranged from 10^4 -10^5 CFU/ml. [48] studied the microbiological assessment of Nile tilapia fingerlings (*O. niloticus*) in glass aquaria for 16 weeks, and they found that, total bacterial count in the water reached its maximum in the sixth week being about 8 $x10^6$ CFU/ml. They also reported that, there were no critical counts, whether in water, or fish tissues. Data in Fig.1 also showed that the values of total bacterial count in surface fish during the 12 weeks in four tanks. During the first four weeks the values of total bacterial count in surface fish somewhat similar, but up the time varied in the four groups.

The values of total bacterial count in surface fish took the lowest values in the first group (C, control) where the values fluctuated between 0.55×10^4 and 2.68×10^4 CFU/ml in W0 (Tilapia fingerling fish which stayed in the glass tanks for one week for acclimatization to laboratory conditions) and W12 (the end of experiment after 12 weeks), respectively. The values of total bacterial count in surface fish in the second group (T1) increased by the time until W10 then decline in W12 being 3.02×10^4 CFU/ml. The minimum values were in W0 being 0.19×10^4 CFU/ml, while the maximum values were in W10 being 4.45×10^4 CFU/ml. The values of total bacterial count in surface fish in the third group (T2) in the first six weeks were higher than first (C) and a second group (T1). These values increased from 0.35×10^4 CFU/ml in W0 to reach its maximum in W12 being 3.53×10^4 CFU/ml. The values of total bacterial count in surface fish in the fourth group (T3) were the highest values comparing with C, T1 and T2. These values took the trend of the second group (T1) where the values increased by the time until W10 then decline in W12 being 4.82×10^4 CFU/ml.

The minimum value was in W0 being 0.5×10^4 CFU/ml, while the maximum value was in W10 being 5.31×10^4 CFU/ml. Our results were less pollution of those obtained from [49]. Who reported that, total bacterial count in fresh fish over 10^5 CFU/g. Data in Fig.1 also show that the values of total bacterial count in the fish intestine during 12 weeks in four tanks. The values fluctuated between 1.5×10^3 and 4.11×10^5 CFU/g for control (C) in W0 and T3 in W12, respectively. The count of total bacterial in the second group (T1, the best treatment in protein efficiency) took the same trend of C, while the values were 1.1×10^3 and 5.28×10^5 CFU/g in W0 and W12, respectively. On the other hand, the count of total bacterial count in the third group (T2) and the fourth group (T3) reached the maximum values being 1.47×10^6 and 1.96×10^6 at the end

of experiment after 12 weeks. These high values of total bacterial count may be due to the fish faeces which accumulate in the tanks after 6 and 8 days, respectively. These results indicated that, group C was the lowest group in the bacterial pollution and group T3 was the highest group in the bacterial pollution. W12 was the most bacterial pollution in the water and fish gut, but it was in W10 in the case of surface fish. Our results were in a good line with [50] who published that, the density of total aerobic bacteria in the intestine of Nile tilapia was about 1.5×10^5 CFU/g. Also [15] reported that, fish intestines harbor more bacterial load ($3.1x10^4$ CFU g^{-1}) than fish gills ($3.4x10^3$ CFU g^{-1}).

Our results were less in microbial load than [46] who study the count of total bacteria from the intestine of *O. niloticus* in an aquarium experiment and they found that, total bacteria counts were $2.1x10^6$ and CFU/ml in the intestine [17]. Reported that the total bacterial count from the intestine of tilapia was $3.8x10^6$ - $58x10^6$ CFU/g, which was higher than that of our study. Also, [51] reported that total bacteria counts in the fish gut growing in water tanks were $6.3X10^6$ CFU/ml. These values were higher than total bacterial counts in our experiments.

3.2.2 The total yeast and fungal count of Nile tilapia during twelve weeks

Data in Fig. 2 showed that, the values of total yeast and fungal count in fish (water tanks, fish surface and fish intestine) during 12 weeks in four tanks. The values of total yeast and fungal count in the water were in the lowest values in tilapia fingerling fish (W0) being 0.11, 0.27, 0.4 and 0.6×10^2 CFU/ml in C, T1, T2 and T3, respectively. These values increased gradually by the time and reached its maximum in W12 in all groups (C, T2 and T3) except T1 which reached its maximum in W10, these values were 3.6, 5.7, 7.3 and 3.6×10^2 CFU/ml, respectively.

Data in Fig. 2 also showed that, the values of total yeast and fungal count in fish surface during the 12 week in four tanks. The values trend of C and T1 were increased by the time until W8 then decline in W10 and W12. The values of total yeast and fungal count in the first group (C) fluctuated between 0.17 and 4.56 $\times10^2$ CFU/ml in W0 and W8, respectively.

The values of total yeast and fungal count in the second group fluctuated between 0.08 and 4.56 $\times10^2$ CFU/ml in W0 and W8, respectively. On the other hand, the trend of values of T2 and T3 were similar, where the values increased by the time until W12. The values of total yeast and fungal count in the third group fluctuated between 0.1 and 6.9 $\times10^2$ CFU/ml in W0 and W12, respectively. Finally, the fourth group also, fluctuated between 19 and 810 in W0 and W12, respectively. In general all treatments got similar values.

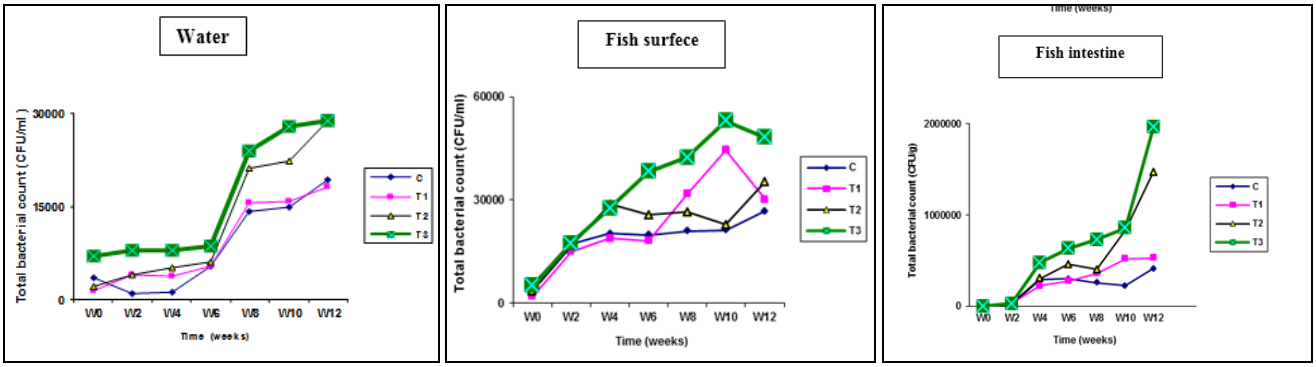

Fig 1: Total bacterial count of Nile tilapia during twelve weeks (CFU) where: (C) (control) the first tank of fish group, which replaced water every two days. (T1) the second group, which replaced water every four days, (T2) the third group, which replace water every six days. (T3) the fourth group, which replaced water every eight days

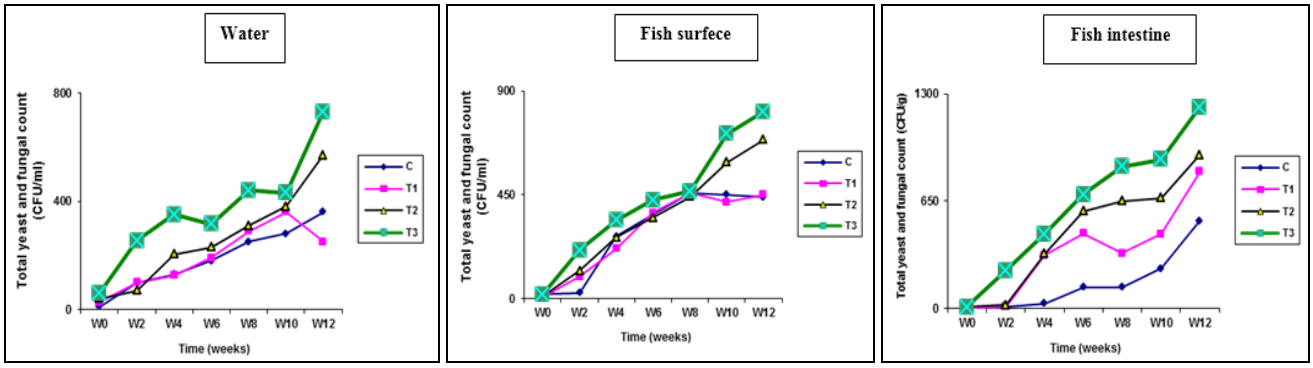

Fig 2: Total yeast and fungal count of Nile tilapia during twelve weeks (CFU) where: (C) (control) the first tank of fish group, which replaced water every two days. (T1) the second group, which replaced water every four days, (T2) the third group, which replace water every six days. (T3) the fourth group, which replaced water every eight days

Data in Fig. 2 also showed that, the values of total yeast and fungal count in the fish intestine during 12 weeks in four tanks. The lowest values were in the first group (control, C) fluctuated between 0.06 and 5.3×10^2 CFU/g in W1 and W12, respectively. Followed by the second group (T1, the best treatment on body weight) where the minimum value being 0.05 $\times10^2$ CFU/g in W1 and the maximum value was 8.3×10^2 CFU/g in W12. By increasing the time and by increasing the

replacing water (every six days, T2 and every eight days, T2) the values of total yeast and fungal count increasing from 9.3 to 12.2 $\times 10^2$ CFU/g by the end of the experiment (12 week), respectively.

These results indicated that, group C was the lowest group in the fungal pollution and group T3 was the highest group in the fungal pollution. W12 was the most fungal pollution in fish surface and fish intestine, but was in W10 in the case of water. [52] Reported that, yeasts can consider a part of the microorganisms in fish gut. In a few cases, yeasts may be more numerous than bacteria, like in the deep-sea. The count of yeast was reached to 10^4 CFU/g. Our results were similar to those obtained from [53] who reported that, the total fungal counts of the fish samples ranged from (1×10^2 to 2.7×10^4 CFU/g). But [54] published that, the total fungal count of the fish samples ranged from $5x10^4$ -$12x10^6$ CFU/g.

3.2.3 The coliform group counts of Nile tilapia during twelve weeks

Data in Fig. 3 showed that the values of the coliform group count in fish (water tanks, fish surface and fish intestine) during 12 weeks in four tanks. It was observed that, the values of coliform group count in fish water tanks groups (C, T2 and T3) increase by the time until W12 except T1 which decline in W12. Generally, the minimum values were in the first group (C) followed by second, third and fourth group (T1, T2 and T3), respectively.

In the first group (control, C), the minimum values of coliform group count in fish water tanks were 0.8, 0.9, 1.02, 1.7, 2.9, 3.3 and 3.9×10^2 CFU/ml in W0, W2, W4, W6, W8, W10 and W12, respectively. By increasing the time of replacing water from 2 day in C into 4 days in T1, these values increased to 0.1, 1.2, 1.35, 1.8, 3.3, 4.7 and 4.6 $\times10^2$ CFU/ml, respectively. When the time of replacing water was after 6 days, these values increased to 0.42, 0.8, 1.97, 2.5, 4, 5.4 and 6.5×10^2 CFU/ml, respectively. In the fourth fish group (T3) the time of replacing water reached its maximum, which was after 8 day, these increasing of the time due to increasing the values of coliform until reached its maximum being 0.4, 0.9, 2, 3.1, 4.4, 6.5 and 7.4×10^2 CFU/ml, respectively. Similar results were obtained from [46] who study the count of total coliform from water of *O. niloticus* in an aquarium experiment and they found that, coliform count was $3.4x10^3$ CFU/ml in water.

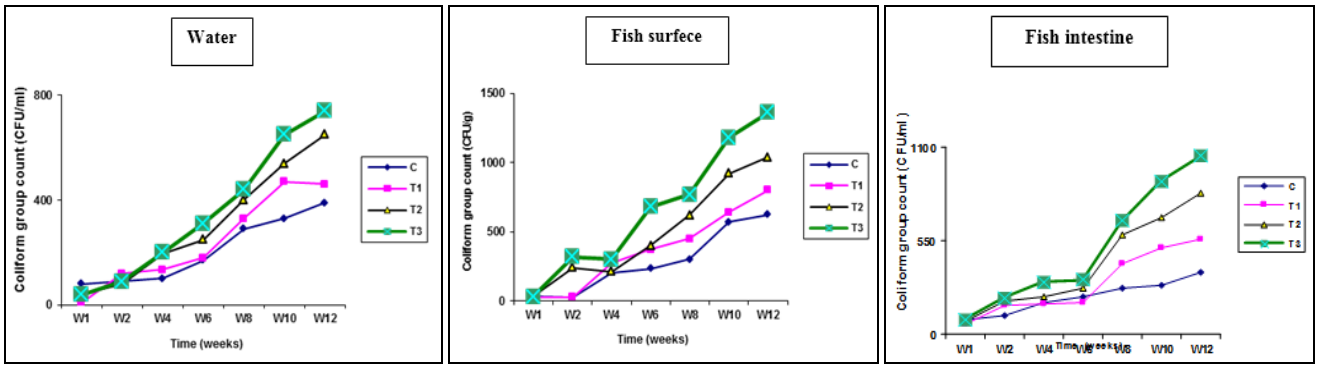

Fig 3: The coliform group counts of Nile tilapia during twelve weeks (CFU) where: (C) (control) the first tank of fish group, which replaced water every two days. (T1) the second group, which replaced water every four days, (T2) the third group, which replace water every six days. (T3) the fourth group, which replaced water every eight days Fig. 3.

Data in Fig. 3 also showed that, the values of the coliform group count in the fish surface during the 12 weeks in four fish tanks. It was observed that, the values of coliform group count in all groups increase by the time until reaching its maximum values in the last week (W12). Generally, the values were closed until the sixth week and then diverged. The values of coliform group count in the control were in minimum being 0.9×10^2 CFU/ml in W0 and gradually increased until W12 being 3.7×10^2 CFU/ml. These values increased to 0.6 and 5.6 $\times10^2$ CFU/ml in T1, respectively. T2 and T3 take the same trend of C and T3 while its values were 0.8 and 8.3×10^2 CFU/ml in the third group (T2) being 0.9 and 10.5×10^2 CFU/ml in the third group (T3), respectively.

Our results were less pollution of those obtained from [49] who reported that, Enterobacteriaceae was found in fresh surface fish, up to $3x10^3$ CFU/g. Data in Fig. 3 also showed that, the values of the coliform group count in the fish intestine during 12 weeks in four tanks. It was observed that, in all fish groups the counts of coliform group increase by the time. The minimum values were in the first week being 0.34, 0.23, 0.31 and 0.3×10^2 CFU/g in T1, T2, T3 and T4, respectively. The maximum values were in the last week (W12) being 6.23, 8, 10.4 and 13.6 $\times10^2$ CFU/g in T1, T2, T3 and T4, respectively.

We can explain these findings by the values presented in Table 1 which show the chemical analysis of experimental diet which fed to Nile tilapia (*O. niloticus*). Where the dry matter, organic matter, crude protein, crude fat and nitrogen free extract were 90.96, 81.31, 35.4, 4.35, and 35.56%, respectively. This rich diet may encourage the microorganism growth, including coliform group.

These results indicated that, group C was the lowest group in the coliform pollution and group T3 was the highest group in the coliform pollution. W12 was the most coliform pollution in surface fish and fish intestine, but was in W10 in the case of water. The concentration of total coliform found in the intestine of Nile tilapia was about 7.5×10^2 CFU/g [50] While, coliforms count from the intestine obtained by [55] was 8.75 $x10^3$ CFU/g of *O. niloticus*, these results somewhat similar to our results. But, unsimilar results were obtained by [46] who study the count of total coliform from the intestine of *O. niloticus* in an aquarium experiment and they found that, coliform count was $5.9x10^5$ CFU/ml in intestine. From the data presented in Figs. 1, 2 and 3, all these experiments indicated that, group C (control) was the lowest group in the microbial pollution and group T3 (which replaced water every eight days) was the highest group in the microbial pollution. W10 was the most microbial pollution in water fish tanks in all fish groups except in bacteria was in W12. While W12 was the most microbial pollution in surface fish in all fish groups except in bacteria was in W10. Finally, W12 was the most

microbial pollution in intestine fish in all fish groups.
These findings could explain by [50] who reported that, fishes are very much susceptible to contamination with different bacteria because of their highly perishable protein content in their body. Coliforms are not the normal flora of bacteria in fish. Due to deposition of human excreta in water is contaminated and when this contaminated water is ingested by the fish, they become contaminated. Fish was contaminated with total aerobic bacteria as well as total coliform, fecal coliform and *E. coli*. This bacterial population was higher in Nile tilapia. This might be due to the contamination of source water or might be due to secondary contamination during the time of handling.

3.3.3 Identification of isolated fungi
Five different colonies of fungal isolates were chosen for identification. The morphological characteristics of colonies in different cultivation media (PDA and RYS) were used. The vegetative and reproduction strictness observed using a light microscope were also considered. The first colony grew rapidly on PDA medium and the conidial heads were typically arranged in zones. The colony color was yellow on PDA medium; the conidiophores appear as powdery mass. Microscopically, the appearances of these conidiophores are granular with pale yellow-brown walls that attach abruptly to a "globose to subglobose vesicle". From these characteristics, these isolates were identified as *Aspergillus ochraceus* following [36].
[56] Reported that, *Aspergillus* was the most common and yielded 52.40% of total fungi. It was represented by 12 species and one variety of *A. flavus* var. *columnaris* (broadest spectrum). Also, [57] reported that, forty-three fungal species were recovered from skin; gills, kidney; liver and intestine of all specimens. The most common genera were *Aspergillus*, *Penicillium* and *Trichoderma*. The second fungal isolate showed that, colony was yellow green on Rose Bengal medium (RYS), conidiophores colorless, long, conidial heads typically radiate and conidia globose to subglobose. From these characteristics following the schemes of [36], this isolate was identified as *Aspergillus oryzae*. Similar results were obtained from [53] who reported that, *A. oryzae* was found at low relative densities among the fungal genera isolated from fish. They also reported that, among the toxigenic genera, the genus *Aspergillus* (68%) was the most prevalent, followed by *Penicillium* species (60%). Other genera were isolated at lower frequencies such as *Wallemia*, *Eurotium*, *Aureobasidium*, *Mucor*, and *Nigrospora* species. The relative density of *Aspergillus* spp.; *A. niger* and *A. flavus* were the most prevalent, followed by *A. versicolor*. *A. fumigatus*, *A. candidus*.
The growth of the third fungal isolate on PDA medium was white, conidial heads dark, brownish black to black reverse colorless, radiate or splitting into several irregular, conidiophores hyaline to brown and smooth-walled. Vesicles globose to subglobose. From these characteristics, this isolate was identified as *Aspergillus niger* according to the protocol of [36, 58] studied the fungal load of five fish's species including Nile tilapia and he found that, the most common isolated fungi was *A. flavus*. Also, he found the following species, *A. clavatus*, *A. ochraceous*, *A. parasiticus*, *A. sydowii*, *A. terreus*, *A. versicolor*, *Penicillium chrysogenum*, *Trichoderma viride*, *Paecilomyces* sp. and *Phoma* sp. The cultural characteristics of the fourth colony were white, dry, powdery to cottony colony on PDA medium. When disturbed on the surface, the colony becomes yeast-like or slimy. Blastoconidia, conidiophores and pseudohyphae were absent. From these characteristics, this isolate was identified as *Geotrichum candidum* following the protocol of [35].
The last colony was rapid grown, flat, filamentous, and velvety, woolly, or cottony in texture on PDA medium. The colony was initially white and become blue green in time. Visualized as globose to elongated sausage-shaped cells that multiply by fission. From these characteristics, this isolate was designated as *Penicillium* sp. [37, 53]. Reported that, *Penicillium* species was the second fungus isolated from fish samples (60%) and the relative density of *Penicillium* spp. was *P. citrinum* which was the most prevalent, followed by *P. glabrum*. Also, [56] reported that, *Penicillium* was represented by 7 species and found in 19 out of 31 samples yielding 11.02% of total fungi. On the other hand [54]. Reported that, *Mucor* spp. and *Rhizopus* spp. (37.5%) were the most predominant fungal isolates, followed by *Penicillum* spp. (18.8%) *Aspergillus*. *spp*. was the least prevalent (6.3%).

4. Conclusion
The results in present study demonstrated that the best nutrient utili-zation parameters were in T1of Nile tilapia *O. niloticus,* those were agreement with microbial activity parameters. We suggested that water renewal after four could improve the fish production and reduced the using water and replacing cost the water in aquaculture production with disturbance little of microbial activity.

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