International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 16-06-2022, Accepted: 02-07-2022, Published: 19-07-2022
Volume 7, Issue 2, 2022, Page No. 1-10

Effect of dietary inclusion of commercial probiotics on growth of Nile Tilapia (*Oreochromis niloticus*)

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Abstract
A total no of 150 Nile tilapia fingerlings weight ranging from 4.06 g to 5.47 g were equally divided into 15 glass fiber tanks of 55L capacity. The total stocking weights were in the range of 48.63 g to 49.8 g. A probiotic (Promarine) was used at 0% (control, treatment 1), 0.5% (treatment 2), 1.0% (treatment 3), 1.5% (treatment 4) and 2.0% (treatment 5) inclusion rates in the experimental diets. The fish Nile tilapia were fed with 30% crude protein. The growth performance and nutrient utilization of Nile tilapia including weight gain, specific growth rate, and daily growth rate were significantly ($P<0.05$) higher in the treatment receiving probiotic than the control diet. The growth was significantly highest in treatment 2 (199.93 g) and treatment 4 (196.86 g). Similarly, treatment 3 (181.8 g) and treatment 5 (175.13 g) were significantly higher than the control diet (160.2 g). Similarly, the specific growth rate was also significantly highest in treatment 2 and treatment 4. The SGR was significantly higher in treatment 3 and treatment 5 than in the control diet. The feed conversion ratio in treatment 2 and treatment 4 was significantly lowest with other treatments but was non-significant with each other. The FCR was small in treatment 3 and treatment 5 than in the control diet. The feed with 0.5% and 1.5% Promarine probiotics inclusion showed significantly better performance in growth parameters as compared with other treatments.

Keywords: Nile tilapia, aquaculture, commercial probiotics, promarine, bacillus SPS

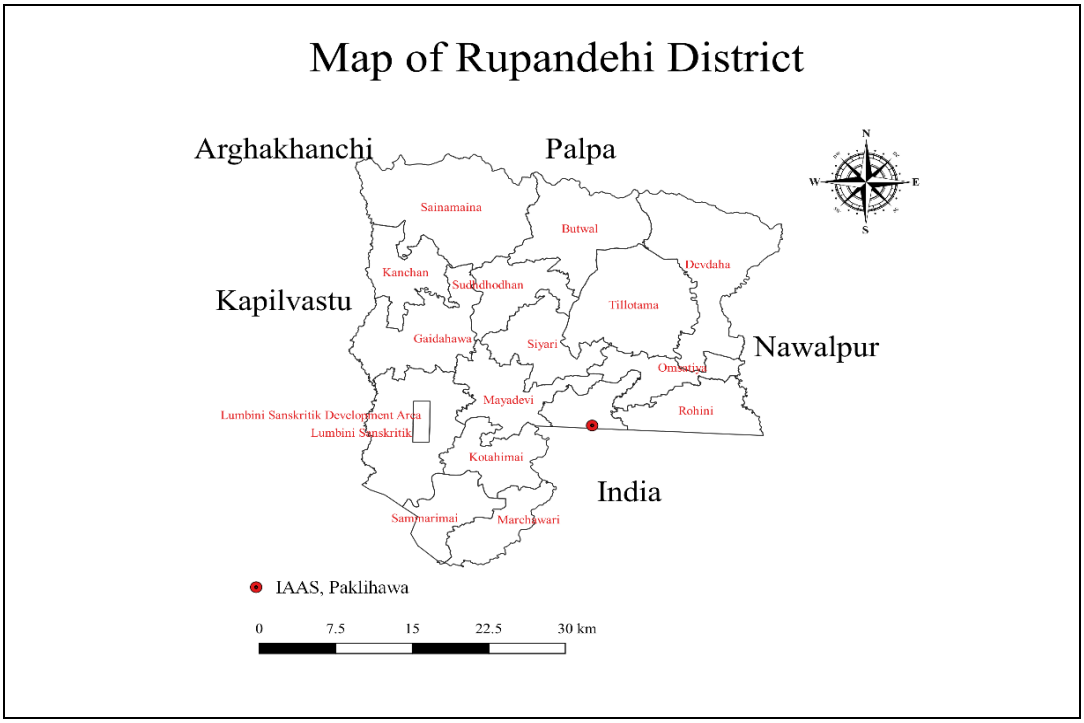
Introduction
The rearing of aquatic organisms under controlled or semi-controlled conditions is known as Aquaculture. Aquaculture is now the world's fastest-growing food production sector generating additional income and employment for a fast-growing population. Annual global fish consumption has increased from 9Kg in 1961 to 20.3Kg in developed countries and 6.1Kg to 12.6Kg in the least developed countries in 2017 (FAO, 2020) [11]. Nile tilapia is an important type of fish, which was caught in Biblical times from the Sea of Galilee. At that time, Tilapia was known as "musht" or now it is known as "St. Peter's Fish". Nile Tilapia belongs to class Teleostomi (bony skeleton), subclass Actinopterygii (single dorsal fin), order Cichliformes, and Family Cichlidae (Shrestha, 2019) [24]. The body is compressed and caudal peduncle depth equal to the length of Nile Tilapia cycloid scales, a knob-like protuberance is absent on the dorsal surface of snout, no sexual dimorphism on upper jaw length, 27 to 33 gill rakers arch are found on the first gill and also Nile Tilapia is also popular for faster growth as compared to other cultured fish and behavior of feeding is omnivorous (El-Sayed, 2006) [10]. Because of its economic importance tilapia is selected for the experimental model worldwide and also has more reasons (Popma and Masseri, 1999). Nile tilapia can survive poor quality of water but could not survive in sustained water temperatures below 10°C and above 42°C (Towers, 2021) [27]. Below 16°C tilapia stop feeding and below 20°C retards the growth of the body (El-Sayed, 2006) [10]. 25°C to 30°C is a suitable water temperature for Nile tilapia (Bhujel, 2000) [3]. Dissolved oxygen level must be in 6.5 to 7.5mg/L to attain quick and proper development of tilapia (Xu *et al.*, 2005). Phytoplankton (blue-green algae and diatoms) are primarily utilized by Nile tilapia but macrophytes are also consumed when blue-green algae and diatoms are low (Bhujel, 2014) [4]. 20 – 45% of protein requirement of tilapia and for improvement in larval quality and performance required 35% crude protein diet (Winfree and Stickney, 1981) [30].
According to (Moriarty, 1997) [17] probiotics are defined as addition of water that modify the sediment and bacterial composition of the water, where the aquatic animals live so therefore improving water quality results on

improving the health of the aquatic animal and removal of pathogens or at least minimizing the effect of pathogens on aquatic animal. By removal of the restriction found improvement on the intestine and improving microbial balance beneficially affects the host animal Gram *et al.* (1999) [12]. Biostart Super™ is a commercial probiotics which shown for significant enhancement in surviva and production of *Ictalurus punctatus* in culture ponds (Queiroz and Boyd, 1998) [21]. Uniformity, growth rate and survival size of marine fish larvae is increased by adding gram positive probiotic bacterium (Blain Kennedy *et al.*, 1998) [5]. According to (Nayak, 2010) [18] the probiotics are effectively regulates immune cells to make cytokines such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β) and transforming growth factor β (TGF-β). *Bacillus amyloliquefaciens* used as a probiotics in the diet, which shows disease resistance and improvement in the immune system of Nile Tilapia (Selim and Reda, 2015) [23]. Increasing a host's defense against diseases, provide better environment for culture and returning a decline microbiota to a normal beneficial status are beneficial effects to the host caused by probiotics (Huynh *et al.*, 2017) [15]. For enhancing the digestion and absorption of nutrients of Nile Tilapia, *B. amyloliquefaciens* are used and also increased villi height and the number of glass cells of the intestine (Silva *et al.*, 2015) [25]. Use of commercialized feeds, meeting faeces of fish, and decay of dead fish are result in the contamination of floor and outside waters in aquaculture. Combinig of sediment vertically into water and more use of chemicals also results in pollution of water (Wang *et al.*, 2018) [19]. The water quality of aquaculture should be improved by exchange of water. This is most ordinary method used in improving the water but it is high cost, require more man power, and also establish the culture systems of pathogens (Devaraja *et al.*, 2013) [8]. Immuno-stimulations are found resistance against pathogens, direct hampering of pathogenic microorganisms, supplying of enzymes and nutrients, better feed utilization and growth are other benefits of probiotics. *Bacillus* are used for improving the quality of water. (Kuebutornye *et al.*, 2019; Soltani *et al.*, 2019) [16, 26]. *Bacillus sps.* plays significant roles in the nitrogen cycle through ammonification (Hui *et al.*, 2019) [14], nitrification (Rout *et al.*, 2017) [22], and denitrification (Verbaendert *et al.*, 2011) [28] as well as nitrogen fixation (Yousuf *et al.*, 2017) [32]. Reduction of nitrogen and phosphorus concentrations, organic matter decomposition, nitrate control, hydrogen sulfide, ammonia and lower disease occurrence are the beneficial effects found on water quality by probiotics in aquaculture (Boyd and Gross, 1998; Cha *et al.*, 2013) [6, 7]. The mixture of *Bacillus* strains (such as *B. subtilis, B. licheniformis and Bacillus pumilus*) was found to maintain TDS within the acceptable range for the culture and increased in pH was also observed in *Bacillus* treated tilapia ponds (Elsabagh *et al.*, 2018) [9].

Materials and Methods

Location site of an experiment

The experiment was conducted from January 2021 to April 2021 at the Aquaculture Laboratory of the Institute of Agriculture and Animal Science (IAAS), Paklihawa campus, Rupandehi for 90 days. The site is located 4km southwest of Bhairahawa located at an altitude of 110m above sea level (Siddharthanagar Municipality, 2021). The geographical location is latitude 27° 28' 48.5"N and longitude 83° 26' 50.2"E. The research was conducted from January to April 2021.



Source: (LGCDP/MOFALD, 2014)

Fig 1: Administrative map showing research site at IAAS, Rupandehi district

Experiment Details

The research was conducted in 15 aquariums of size 1ft × 2ft × 1ft. Commercial feed probiotics were incorporated in the basal feed which was made using locally available feed ingredients. For the experiment, a Completely Randomized Design was used having the five treatments and three replications.

Five experimental treatments are:

- Treatment 1 (Control): containing soyabean meal, rice bran, mustard oil cake and wheat flour.
- Treatment 2: control + 0.5% commercial probiotics
- Treatment 3: control + 1.0% commercial probiotics
- Treatment 4: control + 1.5% commercial probiotics
- Treatment 5: control + 2.0% commercial probiotics

All the treatments were randomized by lottery method.

Collection of Feed Ingredients

For the feed preparation, soybean seed mustard oil cake, rice bran, wheat flour, and vitamin premix were used. Soybean was taken as a protein source, mustard oil cake was taken as both protein and fat source, rice bran was taken as carbohydrate/energy source and wheat was taken as energy and binding agent to bind all the ingredients with each other. Among the ingredients, soybean seed and vitamin premix were bought from a local market in Bhairahawa. Similarly, mustard oil cake, rice bran, and wheat flour were bought from the milling industry in villages near Bhairahawa. Commercial probiotics, Promarine, were obtained from Shrestha Agro Concern in Narayanghat, Chitwan.

Preparation of Feed Ingredients

All the inert materials from soybean seed were removed and heat treatment to remove its anti-nutritional factors. After cooling at room temperature, the soybean seed was ground in a mixture machine. Then, it was sieved with a mesh of 0.5mm size. Mustard oil cake was ground and passed through 0.5mm mesh size. For rice bran and wheat flour, to remove any inert materials, they were also sieved through the same 0.5mm mesh.

Proximate Analysis

For proximate analysis, all the ingredients were weighed 250g each and kept in air-tight plastic bags, and labeled. Those were sent to National Animal Feed and Livestock Quality Management Laboratory, Harihar Bhawan, Lalitpur.

Table 1: Proximate composition of feed ingredients

S.N	Parameter	Soyabean meal		MOC		Rice bran		Wheat Flour	
		MoA	(%)	MoA	(%)	MoA	(%)	MoA	%
1	Moisture	NIRS	7.12	NIRS	7.90	NIRS	9.13	NIRS	7.5
2	CP	NIRS	47.12	NIRS	38.99	NIRS	12.22	NIRS	14.02
3	CF	NIRS	13.43			NIRS	3.93	NIRS	4.59
4	Ash	NIRS	7.71						
5	Others		5.11				9.18		

Source: National Animal Feed and Livestock Quality Management Laboratory Hariharbhawan, Lalitpur, December 05, 2020.

Commercial Probiotics

Commercial probiotics were supplied by the Shrestha Agro Concern, Narayanghat, Chitwan, Nepal. The probiotics, "Promarine" manufactured by Vibro Pharma Private Limited, Mumbai, India was used. According to the manufacturer, the following *Bacillus sps.* were available in the "Promarine".

- *Bacillus amyloliquefaciensBacillus licheniformis*
- *Bacillus megaterium*
- *Bacillus pumilus*
- *Bacillus subtilis*

The total viable spores available in the probiotics were not less than 200 million CFU per gram.

Feed Formulation and Preparation

Pearson's Square method was used for the feed formulation. Crude protein of all the ingredients was taken reference for the feed of 30% CP. All the feed ingredients were mixed thoroughly and the ball was made by adding 200ml water per Kg feed. The ball was then passed through a manually operated pellet machine. A Pellet of 1.5mm size was obtained from the machine which was sun-dried for one week.

Table 2: Amount of feed ingredients per 1Kg of experimental feed

Ingredients	T1 (g)	T2 (g)	T3 (g)	T4 (g)	T5 (g)
Soyabean meal	280	280	280	280	280
MOC	280	280	280	280	280
Rice bran	215	215	210	215	205
Wheat flour	215	210	210	205	205
Vitamin and Mineral Premix	10	10	10	10	10
Commercial Probiotics	0	5	10	15	20
Total	1000	1000	1000	1000	1000

*Vitamin mineral premix/Kg contains the following: VitaminA 7,00,000I.U, Vitamin D3 70,000I.U, Vitamin E 250mg, Cobalt 250mg, copper 1200mg, Iodine 325mg, Iron 1500mg, Magnesium 6000mg, Potassium 100mg, Sodium 5.9mg, Manganese 1500mg, Sulphur 0.72%, Zinc 9600mg, DL-Methionine 1000mg, Calcium 25.5%, Phosphorus 12.75%.

Table 3: Proximate composition of treatments

Parameters	MoA	T1 (%)	T2 (%)	T3 (%)	T4 (%)	T5 (%)
Moisture	NIRS	11.73	11.66	11.46	11.56	11.52
CP	NIRS	30.67	30.12	30.16	30.48	30.55
CF	NIRS	5.92	5.85	5.8	5.85	5.9
Ash	NIRS	9.43	9.53	9.43	9.5	9.45
Fiber		2.07	2.14	2.12	2.15	2.1

Source: National Animal Feed and Livestock Quality Management Laboratory Harihar Bhawan, Lalitpur, January 5, 2021.

Transportation of Fingerlings and Acclimatization

Viable fingerlings of Tilapia size (3-4g) were collected from the Shanti Matshya Hatchery, Bhagalapur, Rupandehi. Fingerlings were transported with proper handle and care. No mortality of fingerlings was observed during the transportation. All the fingerlings were kept in a separate tank for acclimatization and allowed to acclimatize for 14 days before the start of the experiment in research aquaria. During the acclimatization period feeding of extruded commercial feed having crude protein, 28% was done before using experimental diets.

Research Setup

All the types of equipment that were used in the research work were sanitized using potassium permanganate solution and sun-dried. The equipment was also used sanitized with a salt solution of 25g crystal salt per liter of water. The salt solution was also used in cleaning aquariums. Air compressor pipes with air stones were established in each aquarium. The continuous power supply was ensured for the air compressor by connecting the battery inverter.

Stocking

Stocking was done after 14 days of acclimatization in a tank. Before stocking, fingerlings were dip bathed in a 2ppm solution of KMnO4 for 5 minutes. The individual weight of fingerlings was taken and then transferred to the aquariums. The individual weight of fingerlings was between 4 to 5g. The stocking density was 10 fish per aquarium tank. The total weight of fingerlings per tank was between 48g to 50g. Proper handling and stocking of fingerlings were done and no mortality of fingerlings was observed due to stress.

Feeding

Fingerlings were hand-fed with prepared pellet feed following the total body weight of the fishes of each aquarium. Feeding was done two times a day, at 8 AM and 4 PM. Before feeding, the prepared pellet feed was grinded in a mortar pestle to make small size particle that makes it easier to swallow for fish. Fish were fed 10% of their body weight for the first month and were adjusted to 7.5% and 5% in the following months.

Cleaning and Exchanging Aquarium Water

The water of all the aquariums was changed daily. The water was thrown out using a siphon pipe leaving1/3rd water. Fresh and clean water was added again into the tank. The temperature difference of water was ensured to be less than 1°C. While removing the water, all the faces and unfed feed were all removed. It was ensured, to make the water in the tank as clean as possible.

Water Quality Parameters

Dissolved Oxygen (DO), pH, and temperature were measured every day. A portable pH meter (Hana instrument, accuracy ± 0.1) was used to monitor pH. Likewise, a thermometer was used to measure water temperature. Similarly, a dissolve oxygen meter (Lutron PDO-519) was used to measure dissolved oxygen. The temperature in degree centigrade (ºC), dissolved oxygen in parts per million (mg/L) was noted for each value monitored.

Moreover, another water quality parameter total ammonia nitrogen was measured weekly using API freshwater master test kit.

Procedure for Ammonia Test:

- 5ml water sample in 50ml sample test tube with the help of 5ml syringe was taken.
- 8 drops of reagent I was added in to the sample water.
- 8 drops of reagent II was added in to the sample water.
- The cap of test tube was closed and shaked vigorously for 5 seconds.
- After 5 minutes the colour was compared with the Ammonia color chart and data was recorded.

Data Collection Protocol

Monthly from each aquarium entire fish was collected using scooping net and its growth performance was assessed. The weight of individual fish were recorded using electronic compact scale (Kerro series P3 BL5002 Max-500g, D=0.01g).

Growth Parameters

- Mean Weight Gain (MWG) (g): Final Mean Weight (g) – Initial Mean Weight (g)
- Specific Growth Rate (%/day): [[log (final weight) (g)] – [log (initial weight) (g)] / time interval in days] × 100
- Feed Conversion Ratio (FCR): Dry weight of feed gain (g) / Wet weight gain (g)
- Daily weight gain(g/day): Average final weight (g) – Average stocked weight (g) / culture period
- Survival Rate %: (total number of fish harvested / total number of fish stocked) × 100

Harvesting

Final harvesting of Tilapia fish was done after 90 days in a research aquarium. The fish were taken out with the help of a scooping net. The individual weight of fish in each aquarium was recorded using an electronic Compact scale (Kerro series P3 BL5002 Max-500gm, D=0.01g). The data was collected over time and based on individual fish observations. The population means for each growth parameter was computed. The analysis of variance was used to compare different growth parameters using R-Stat. The mean and standard errors were calculated for each treatment. The data entry was done through MS Excel 2007. The accepted level of significance was $p<0.05$.

Results

Growth parameters

The stocking weight (SW), average stocking weight (ASW), harvest weight (HW), average harvest weight (AHW), weight gain (WG), survivability rate (SR), daily growth rate (DGR), specific growth rate (SGR) and feed conversion ratio (FCR) of five different treatments are presented in Table 4.

From the table, it is clear that stocking weights per aquarium which were as low as 48.63g and as high as 49.8g were non-significant with each other's. Similarly, the average stocking weight was also not significant. At the termination of experiment total harvest weight of fish was highest in T2 (199.93±0.02g) which was not significantly different with T4 (196.86±0.00g) but were significantly higher than T3 (181.80±0.01g), T5 (175.13±0.01g), T1 which was 160.2±0.03g. Likewise, T3 and T5 were also significantly different than T1 ($p<0.05$) but were not significantly different with each other ($p>0.05$). Similar was the case with average harvest weight. Highest mean harvest weight was recorded in T2 (19.99±0.22g) ($p<0.05$) which was not significantly different with T4 (19.68±0.28g) ($p>0.05$) but were significantly higher than T3 (18.18±0.41g), T5 (17.5.51±0.25g) and T1 (16.02±0.48g) at ($p<0.05$).

Significant differences in food conversion ratio (FCR) was observed among treatments during the study period ($p<0.05$). Lowest FCR was recorded in T2 (2.73±0.05) which was not significantly different from T4 (2.85±0.03) but was significantly different from T3 (3.15±0.10), T5 (3.26±0.04) and T1 (3.61±0.11) ($p<0.05$). Highest FCR was seen in T1 (3.61±0.11) which was significantly different from other treatments ($p<0.05$). However, there were no significant difference in FCR between T3 (3.15±0.10) and T5 (3.26±0.04) ($p>0.05$) but were significantly different from T1 (3.16±0.11) ($p<0.05$).

In a like manner, there was a significant difference ($p<0.05$) in specific growth rate (SGR). Highest SGR was observed in T2 (1.57±0.01%/day) which was not significantly different with T4 (1.53±0.01%/day) ($p>0.05$) but were significantly different with T3 (1.46±0.02%/day), T5 (1.40±0.01%/day), T1 (1.31±0.03%/day) ($p<0.05$). T3 was non-significant with T5 ($p>0.05$) but both were significantly different with T1 at $p<0.05$. Likewise, highest daily weight gain (DWG) was observed in T2 (0.17±0.00g/day) which was not significantly different with T4 (0.16±0.00g/day) ($p<0.05$) but were significantly different with T3 (0.15±0.00g/day), T5 (0.14±0.00g/day), T1 (0.12±0.00g/day) ($p<0.05$). T3 was non-significant with T5 ($p>0.05$) but both were significantly different with T1 at $p<0.05$.

Similarly, Highest weight gain was observed in T2 (151.30±2.45g) which was not significantly different with T4 (147.06±2.82g) ($p>0.05$) but were significantly different with T3 (133.03±4.23g), T5 (125.80±2.55g), T1 (111.10±4.90g) ($p<0.05$). T3 was non-significant with T5 ($p>0.05$) but both were significantly different with T1 at $p<0.05$.

Table 4: Mean value of growth parameters of Nile tilapia during experimental period of 90 Days

Treatment		Growth Parameters							
	SW	ASW	HW	AHW	WG	SR	DGR	SGR	FCR
T1	49.1±0.30	4.91±0.03	160.2±0.03^{c}	16.02±0.48^{c}	111.10±4.90^{c}	100±0.00	0.12±0.00^{d}	1.31±0.03^{d}	3.61±0.11^{a}
T2	48.63±0.26	4.86±0.02	199.93±0.02^{a}	19.99±0.22^{a}	151.30±2.45^{a}	100±0.00	0.17±0.00^{a}	1.57±0.01^{a}	2.73±0.05^{c}
T3	48.76±0.13	4.87±0.01	181.80±0.01^{b}	18.18±0.41^{b}	133.03±4.23^{b}	100±0.00	0.15±0.00b^{c}	1.46±0.02^{b}^{c}	3.15±0.10^{b}
T4	49.80±0.05	4.98±0.00	196.86±0.00^{a}	19.68±0.28^{a}	147.06±2.82^{a}	100±0.00	0.16±0.00^{a}^{b}	1.53±0.01^{a}^{b}	2.85±0.03^{c}
T5	49.33±0.12	4.93±0.01	175.13±0.01^{b}	17.51±0.25^{b}	125.80±2.55^{b}	100±0.00	0.14±0.00^{c}	1.40±0.01^{c}	3.26±0.04^{b}
LSD (= 0.05)	1.62	0.16	10.89	1.09	11.14	-	0.01	0.08	0.25
CV (%)	0.69	0.69	3.28	3.28	4.58	-	5.47	2.86	4.39
Significance	Ns	Ns	***	***	***	Ns	***	***	***

T1= Control Diet; T2= 0.5% Probiotics; T3= 1.0% Probiotics T4= 1.5% Probiotics T5= 2.0% Probiotics

Growth Trend

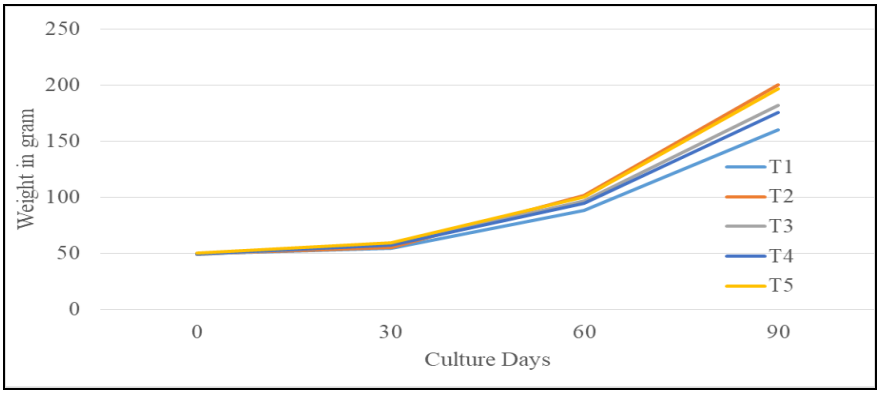


Fig 2: Monthly trend of growth in different treatments during the experimental periodIn the first month of stocking, the increase in growth was negligible in all the treatments. There was some good positive increase in the second month. In the second month, the variation among the treatments was observed. The growth only increased in the last month of the experiment. The variation among the treatment was also significant.

Water Quality parameters

Temperatures

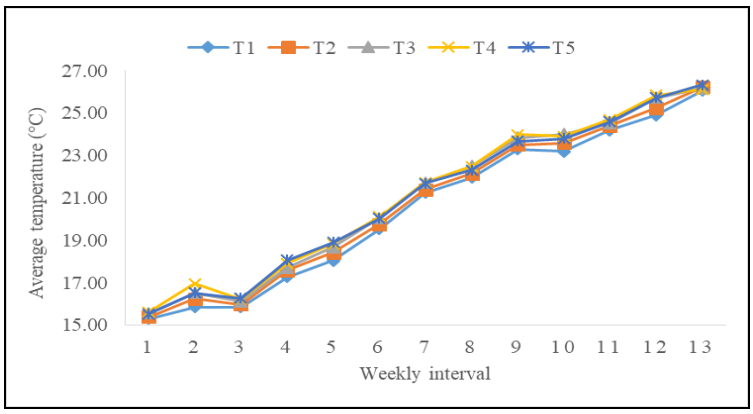


Fig 3: Weekly trend of average temperature in different treatments during experimental period According to the figure, in first three weeks the average water temperature was below the threshold temperature for Nile tilapia. Tilapia stops feeding below 16°C (El-Sayed, 2006) [10]. The temperature in Bhairahawa was cold even in few weeks of February. Due to this reason the growth was very low in the fish. There was more feed wastage in those days. After 4th week of the research setup, the temperature gradually started to increase with little fluctuation in later days. The average maximum temperature experienced during the research period was on the last day of the research which was 26.5°C.

pH

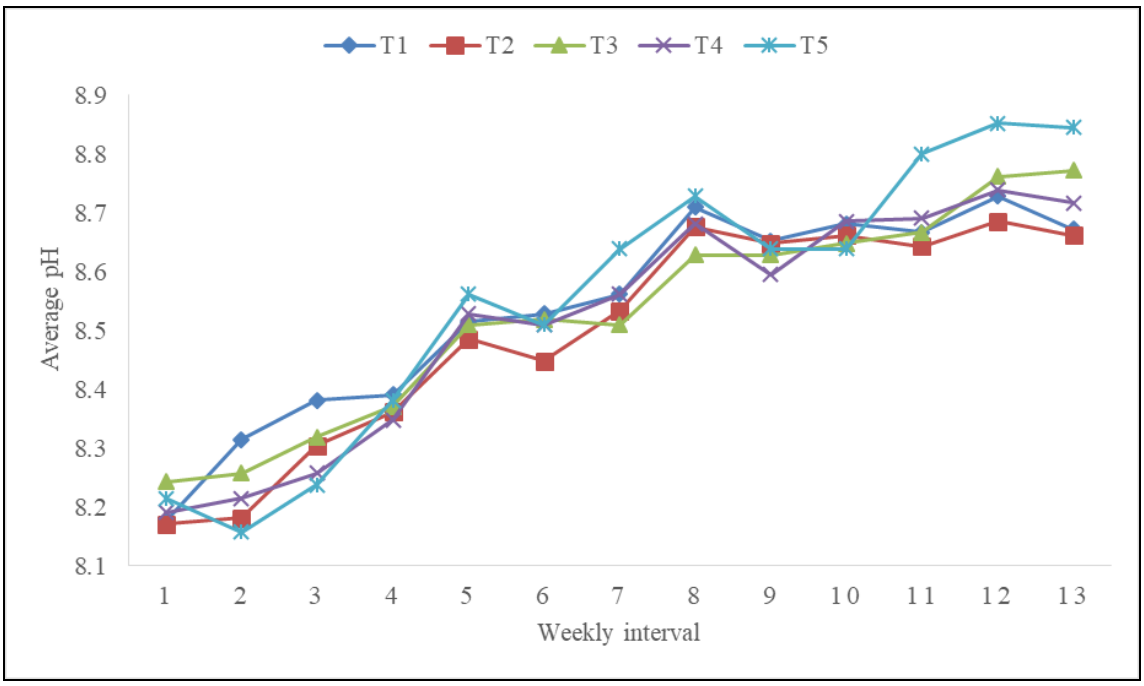


Fig 4: Weekly trend of average pH in different treatments during experimental period The figure shows the average pH of the all five treatments. During the whole experiment period, the pH was in between the optimum level. The maximum pH recorded was below highest threshold which is 9. While, the lowest pH recorded was above 8. This is the optimum pH for fish culture. The pH gradually increased in the initial weeks and slight fluctuation observed during 6th and 9th weeks. During the research, highest pH was recorded in Treatment 5 (2% Promarine) which was still below 9.

Dissolved Oxygen

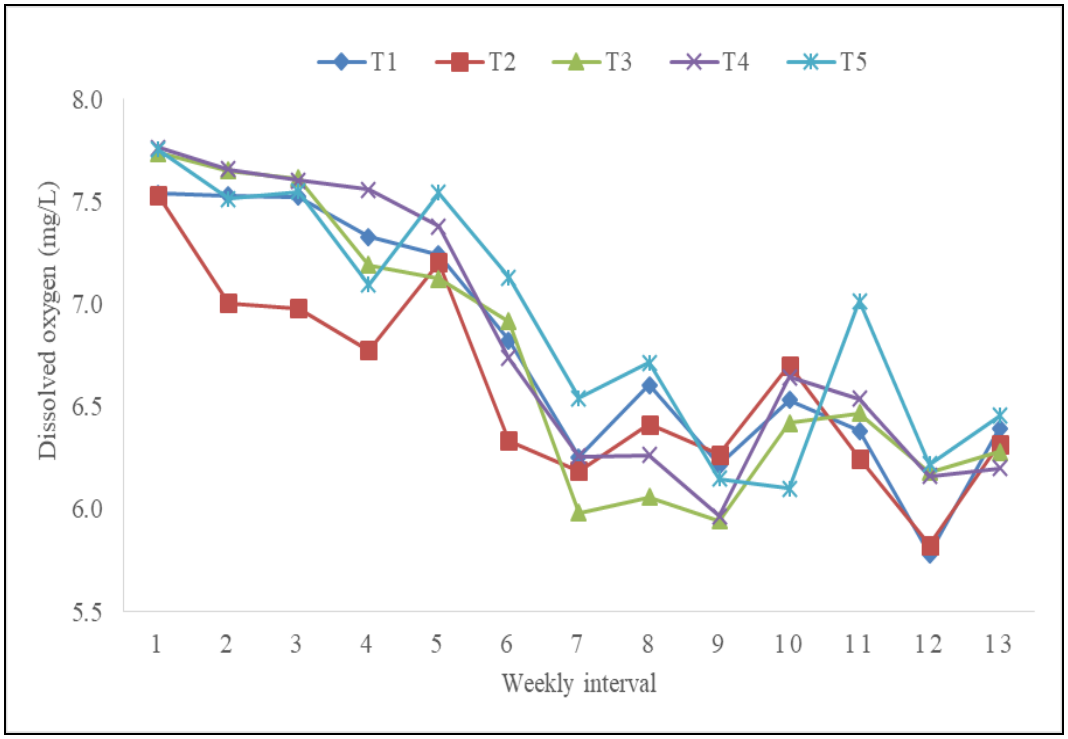


Fig 5: Weekly trend of average dissolved oxygen in different temperature during experimental period According to the figure, the dissolved oxygen level was above the low range of dissolved oxygen. The dissolved oxygen level was above 5mg/L. The dissolved oxygen level was slightly declined as the research period. However, the level difference was not more than 1mg/L among treatments. Treatment 5 recorded sharp fluctuations in a single week but it was not more than 1mg/L. During the research period, the difference in highest dissolved oxygen level to the lowest dissolved oxygen level was near to 1.5mg/L.

Ammonia

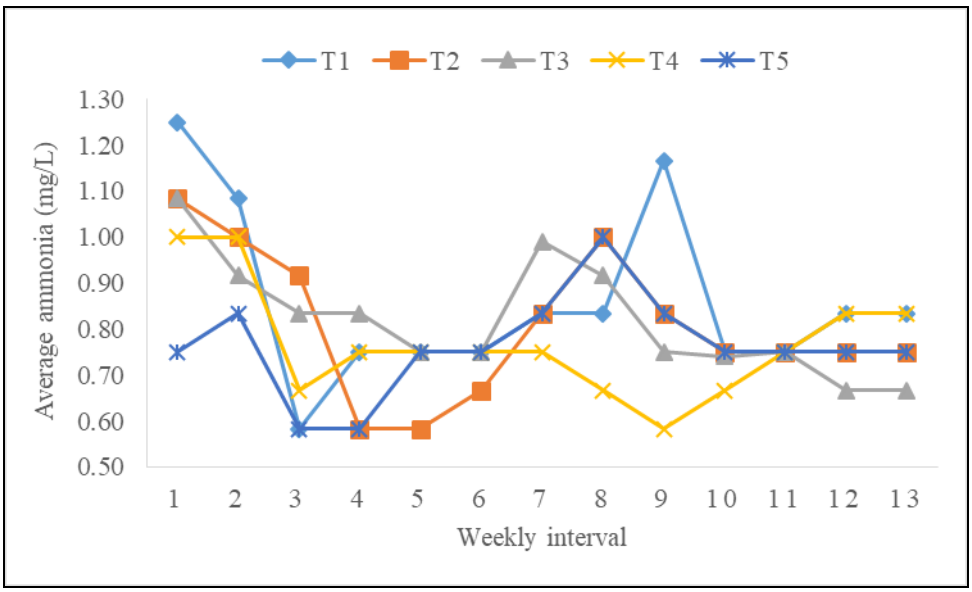


Fig 6: Weekly trend of average ammonia in different treatments during experimental period The ammonia level gradually decreased from the first week. However, the ammonia level slightly increased after 6th week of research setup. Control recorded highest fluctuations, the level exceeds 1mg/L few times. But most of the time it was also below 1mg/L. However, other treatments never exceeds 1mg/L. Ammonia level was lowest in teatment 2 (0.5% Probiotics).

Discussion

In this study, all probiotics-supplemented diets resulted in higher growth in Nile tilapia than the control diet, suggesting that the adding of probiotics enhance in performance of the growth and utilization of food and mitigates the effects of stress factors. (Noh *et al.* 1994) [19] reported the effects of augmenting common carp *Cyprinus carpio* with different additional feed, including antibiotics like bacteria (*Streptococcus faecium*) and yeast (*Saccharomyces cerevisiae*). Dietary inclusion of *Bacillus sps.* Significantly increase the growth in Nile Tilapia (Selim and Reda, 2015; Abarike *et al.*, 2018) [23, 1]. The total harvest weight in T2 (0.5% probiotics) was the highest (199.93±0.02g) among the treatments. (Balqadi *et al.* 2015) [2] found a similar result while using the Promarine in Indian white shrimp.

Adding probiotics is also shows improvement in digestibility for increasing growth performance, which shows for finding better growth with supplementary diets. Probiotics are improving the diet of protein and energy utilization of Nile tilapia and also contribute to the use of protein for better growth. Fish yield of Nile tilapia-fed diets supplemented with 0.5%, 1%, 1.5% and 2% Promarine resulted in 36.04%, 19.80%, 32.37% and 13.23% more production than the control diet respectively (Table 3). Specific Growth Rate was highest in treatment 2 (1.57±0.01%/day), and significantly lowest in treatment 1 (1.31±0.03%/day) among the probiotics included treatments. During the experimental period, the lowest dissolved oxygen level observed was 5.8mg/L. To attain quick and proper development the dissolved oxygen level must be 6 to 7.5mg/L. The effect of ammonia on tilapia performance is also related to water pH, DO, and exposure period. pH and DO levels are not affected by high ammonia concentration (Hargreaves and Kucuk, 2001) [13].

Summary

Most of the fish farmers in Nepal do not use commercial feed. They rely on the locally available feed and feed ingredients. For fish feed most of the farmers use rice bran and mustard oil cake only. However, they use plenty of farm yard manures. Semi-intensive fish farming is practiced in Nepal. Few innovative farmers have started biofloc technology but that is not successful like in neighboring countries. Probiotics are the beneficial micro-organisms for all animals. Inclusion of probiotics enhances growth, reduce feed conversion ratio, increases immunity to the fish.

As there is negligible work on probiotics in Nile tilapia, a research was conducted in Institute of Agriculture and Animal Science, Paklihawa Campus. The research was setup in aquaculture laboratory. The research was aimed of optimizing dose of commercial probiotics. There were five treatments and three replications in the research. Thus, 15 aquaria were selected and the experiment was completely randomized design. All the aquaria were of equal size (1ft × 2ft × 1ft). The commercial probiotics was incorporated in different doses. “Promarine” was used as commercial probiotics. The compostion of probiotics were *Bacillus subtilis, B. licheniformes, B. amyloliquefaciens, B. megaterium and B. pumilus.* The 30% CP feed was made using locally available feed ingredients like soyabean meal, mustard oil cake, rice bran and wheat flour. To enhance the quality of feed Agrim Forte was used as vitamins and mineral supplements. The treatments were T1 (control: basal dose), T2

(basal dose + 0.5% probiotics), T3 (basal dose + 1% probiotics), T4 (basal dose + 1.5% probiotics) and T5 (basal dose + 2% probiotics). Nile tilapia fingerlings of 4g to 5g size were used for the research. 10 fingerlings were kept in 45L water in the aquaria. Feed was given two times daily for 90 days.

After the research, the highest harvesting weight was observed in T2 (199.93±0.02g), similarly highest average weight (19.99±0.22g), highest weight gain (151.30 ±2.45g), highest daily growth rate (0.17±0.00g) and highest specific growth rate (1.57±0.01g) were recorded in T2. While lowest values were recorded in control diet (T1). Second best value was observed in T4 followed by T3 and T5. Likewise, feed conversion ratio was lowest in T2 (2.73±0.05g) and highest in T1 (control).

Conclusion

The use of medication has disturbed the water ecosystem. In contrast to all these problems, probiotics are developed and practiced. Many researchers have found the positive response of using probiotics in disease-resistant and immunity against the pathogen in fishes. Probiotics are also found to increase weight, body growth, daily growth rate, and decrease feed conversion ratio. The research entitled: "Dietary inclusion of commercial probiotics in growth performance of Nile tilapia (*Oreochromis niloticus*)" has added one plus point in using probiotics in fish feed. Commercial probiotics "Promarine" which contains probiotics like *Bacillus subtilis, B. licheniformes, B. amyloliquefaciens, B. megaterium, and B. pumilus* has enhanced the growth and decreased feed conversion ratio. These probiotics when used in a small amount (5g per Kg feed) gives better result when compared with other higher doses. Commercial probiotics "Promarine" increases immunity against fish pathogens and maintains water quality parameters when used 0.5% in feed. The 500g "Promarine" which costs about NPR 1,000 is enough for 100Kg feed. Thus, the use of Promarine probiotics is economical too.

Author Contributions

Conceptualization, writing a research paper, calculation, and analysis made by Ashish Chaudhary; removal of plagiarism, format preparation, grammer checking, and editing made by Aman Kumar Gupta; Supervision under Shailesh Gurung and Suraj Kumar Singh; helps in data collection and analysis made by Nabin Chalaune.

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