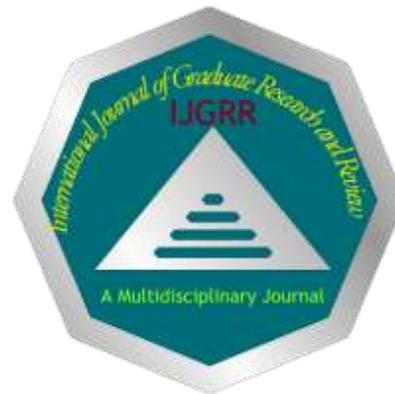


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Effect of Probiotics on the Growth and Food Utilization of Walking Catfish (*Clarias batrachus*) under Laboratory Condition of IAAS, Paklihawa

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Abstract

The experiment was conducted in Aquaculture lab of IAAS, Paklihawa, Rupandehi district from 6th February 2018 to 6th April 2018. The research was conducted for the evaluation of the effect of different probiotics on growth and feed utilization of Catfish (*Clarias batrachus*) under laboratory condition. One hundred and sixty eight catfish fries with average initial weight 1.60-1.70gm were stocked in 12 glass aquarium tank of size 12”*24”*12” each half-filled with water and aerated continuously using an air compressor. The stocking density of catfish fries was 14 fish per aquarium. The experiment was performed at completely randomized block design with 4 treatments and 3 replications. The treatments were T1 Control (Rice bran + MOC), T2 (Rice bran + MOC + Microguard), T3 (Rice bran + MOC + Nicoli) and T4 (Rice bran + MOC + Grozyme). Probiotics i.e. Microguard, Grozyme and Nicoli was each applied at the rate of 2 % of total weight of supplementary feed (Rice bran and MOC) in T2, T3 and T4 respectively. Feed was allocated to each aquaria at the rate of 8 % of total body weight of fish. Water quality parameters pH, DO and Temperature was measured on weekly basis and Ammonia, Nitrate and Nitrite was measured fortnightly. The average pH varied from 8.1 to 8.4 within 2 months while the DO varied from 5.4 mg/l to 7.9 mg/l. The temperature was found optimum ranging from 19.6°C to 24.1°C during the experimental period. Mean nitrate concentration ranged from 0.1 to 2.67 ppm concentration while mean nitrite level ranged from 0ppm to 3.5ppm and the mean ammonia level range from 0.21 ppm to 5.33 ppm. The mean total harvest weight of T2 (Feed mixed with Probiotics/Microguard) was found highest with 39.66 gm seemed fairly high in comparison to the other treatments during the experimental period. Considerably, the mean individual harvest weight in T2 was also found highest with 2.99 gm among other treatments. Moreover, Specific Growth Rate (SGR) of catfish in T2 during the experimental period was also display the best performance with 22.86 than other treatments. Hence Probiotics i.e. Microguard has good performance in terms of growth rate and feed utilization than other probiotics.

Keywords: Mustard Oil Cake (MOC); Walking catfish; Probiotics; Microguard; Nicoli; Grozyme

Introduction

Broadly Aquaculture implies the farming of aquatic organisms including both aquatic animals (fish, molluscans, crustaceans) and aquatic plants (sea weeds, freshwater macrophytes) (FAO, 1988). No doubt, Fish farming in Nepal is pre-dominantly subsistence type in Nepal. Commonly Carps fishes are produced in southern plain part of Nepal. Still it is deeply felt that Fish farming is traditional

activity in Nepal but nowadays commercial fish farming is also practiced in hills and terai of Nepal. Catfish (*Clarias batrachus*) is commonly known as walking catfish. It is named after its ability to walk across the dry land for survival and food. *Clarias batrachus* is native to South-east Asia. Catfish is hardy, opportunistic feeder and can survive months without food. Catfish lives on wide variety of habitats mostly on muddy and stagnant water, rice fields etc. *C. batrachus* has been described as a benthic, nocturnal,

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tactile omnivore that consumes detritus and opportunistically forages on large aquatic insects, tadpoles and fish. Basically Nepalese fish farming depends on natural productivity of fish pond (Gurung, 2003). Feed manufacturer are very limited in Nepal. The purpose of commercial fish farming is to increase the productivity by increasing the weight of the individual fish. The weight gain of fish is obtained by the addition of fish additives in the fish feed. Feed additives result in maximum growth of the fish in relatively short time. For such purpose many studies shows the use of probiotics can be helpful. For decades there has been use of many antibiotics, hormones and other substances for controlling diseases in fish and for increasing the fish weight. This has ultimately led to the emergence of drug resistance bacteria and production of toxic substance in environment and water resources. This creates threat to aquatic life. The use of antibiotic and hormones is to be replaced with some eco-friendly and cheaper substances. Research claim the replacement can be made with probiotics. Probiotics is a relatively new term and can be described as beneficial microorganisms. Probiotics is a living microorganism that beneficially affects the intestinal tract of host and improves the microbial balance (Fuller, 1989). It can also be define as a viable micro-organism which when ingested through the oral cavity in a sufficient quantity confer on the host a beneficial effect due to an improvement in the intestinal microbial balance (Giorgio et al., 2010).

Materials and Methods

The research was conducted in Fish laboratory of Institute of Agriculture and Animal Science, Paklihawa Campus, Tribhuvan University. The duration of the study was 60 days with catfish fries were stocked on 12 glass aquaria tanks each of size (24" × 12" × 12") each half-filled with water were aerated continuously using an air compressor. One hundred and sixty-eight catfish fries with average initial weight of 1.53 to 2.08 were allocated in each aquarium at the rate of 14 fish per tank. The fish fries were carried from Mandal Fish Hatchery located at Pathardada, Rupandehi. Water temperature, dissolved oxygen and pH were recorded weekly. Other water quality parameters (nitrate, nitrite and ammonia) were recorded in every 15 days. Water was changed on every 4 days leaving 1/4th quantity of the previous one at the bottom of aquarium. Rice bran and Mustard Oil Cake (MOC) was mixed at 1:1 ratio whereas probiotics were mixed at 2 % of total weight of supplementary feed.

Research Design

The experimental design was completely randomized design with four treatments and three replications each (Table 1).

- 1) T1- Fish fed with local feed (Control)
- 2) T2- Fish fed with Probiotics 1(Microguard)

- 3) T3- Fish fed with Probiotics 2 (Nicolli)
- 4) T4- Fish fed with Probiotics 3 (Grozyme)

Table 1: Experimental design of research

Treatment	T1	T2	T3	T4
R1	T1R1 (1)	T2R1 (2)	T3R1 (3)	T4R1 (4)
R2	T1R2 (5)	T2R2 (6)	T3R2 (7)	T4R2 (8)
R3	T1R3 (9)	T2R3 (10)	T3R3 (11)	T4R3 (12)

(Note: The number inside the brackets denote the number of aquarium tank during the experiment)

Firstly, Mustard oil cake was soaked overnight in water. Equal weight of mustard oil cake and Rice bran was weighted and mixed thoroughly. The mixture was then oven dried for 6 min at medium temperature. Probiotics was then added to oven dried mixture at the rate of 2% of the mixture. The prepared feed were fed at 8% of their body weight per day between 8:00 -9:00. All fish were weighed and counted fortnightly and feeding rates were adjusted accordingly. The daily measurement of Dissolved oxygen and temperature with Lutron Oxygen meter Model DO 5510 and pH of water with Lutron pocket type Model pH-201 was conducted at 8:00-9:00am. Similarly, Nitrate, Nitrite and Ammonia were measured fortnightly with API test kits.

The fish sampling was done fortnightly by using electronic balance, scoop net and plastic bucket. By the help of scoop net the fishes were caught and placed in the plastic bucket and then weighted fishes on the electronic balance and recorded accordingly. At last, Fish harvesting was done on 6th April 2018 (60 days) of stocking.

Following calculations were done for the growth analysis of the fish:

- Average weight of the individual fish species = Total weight of the individual fish species (Kg)/Total no. of individual fish
- Specific Growth Rate (SGR) (% / day) = $\{(\ln \text{ Final body weight} - \ln \text{ Initial body weight}) / \text{days}\} * 100$
- % Weight gain = $(\text{Final body weight} - \text{initial body weight} / \text{Initial body weight}) * 100$
- Feed conversion ratio (FCR) = Food fed (g dry weight) / Live weight gained (gm)
- Average daily growth rate (ADGR) = $\text{Final total wt of the fish} - \text{Initial total wt of the fish} / \text{Total no. of fish}$
- Feeding rate per fish biomass (gm) = $\text{Total no. of fish} * \text{avg. individual fish wt} * \text{feeding rate} \% / 100$
- Survival rate = $\text{Total no. of fish} / \text{remaining fish} * 100$

Data entry and Analysis

Tabulation of all the data was done in Microsoft Excel computer program. Data was analyzed in Microsoft Excel and SPSS. Microsoft Word was used for preparation of Report.

Result

Weekly parameters were noted from 6th February to 26th March. pH value range from 8.1 to 8.4 during the experimental period (Fig.1). Maximum pH value noted was 8.4 in T1, T2, T3, T4 on 6th February, 27th February, 13th March and 20th March respectively. Similarly Minimum pH value was found to be 8.1 in T1 on 20th February, 6th March and T2 on 13th February, 6th March and 20th March, T3 on 6th February, 20th February, 6th March, 27th March and T4 on 20th February and 27th March respectively. The value of Dissolved Oxygen (DO) ranged from 5.8mg/l to 7.9mg/l (Fig. 2) during the experimental period. The maximum DO noted was 7.9mg/l on 6th February and minimum DO noted was 5.4 mg/l in T3 on 27th March. Furthermore, Temperature was found ranging between 19.6°C to 24.1°C in between 6th February to 27th of March where maximum and minimum temperature was noted in 24.1°C in T1 and 19.6°C in T2, T3, T4 respectively (Fig. 3).

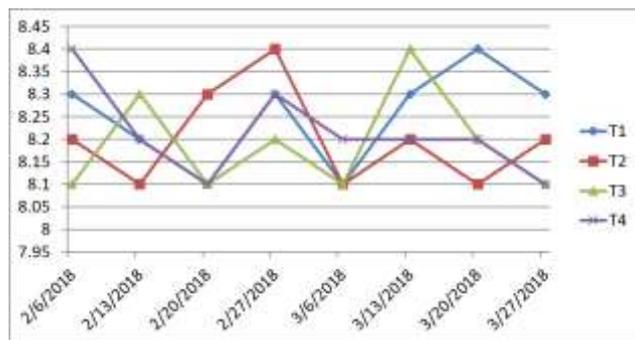


Fig. 1: Mean weekly pH value of different treatments during the experimental period

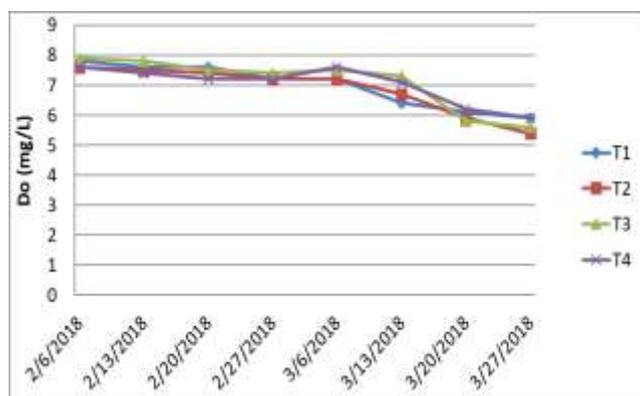


Fig. 2: Weekly mean DO of different treatments during the experimental period.

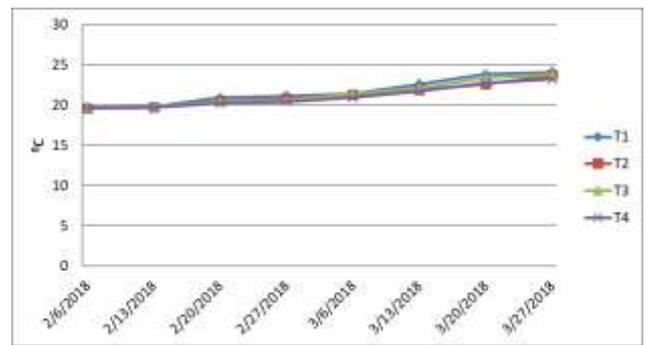


Fig. 3: Weekly mean temperature of different treatments during the experimental period.

Hence, Fortnightly water quality parameters were also measured where Maximum and minimum nitrate level was found 2.67 ppm in T1 on 12th march and 0.1 ppm in T1 on 24th January respectively (figure 4). Accordingly, The mean nitrite level ranged from 0 ppm to 3.5ppm (Fig. 5) during the experimental period. Maximum and minimum nitrite level was 3.5 ppm in T1 on 28th March and 0 ppm in T1, T2, T3 and T4 on 26th February respectively. Furthermore, Mean ammonia level range from 0.21 ppm to 5.33 ppm (figure 6) during the experimental period. Maximum ammonia level was 5.33 ppm in T1 on 28th March and minimum ammonia level was 0.21ppm in T1 and T3 on 24th January.

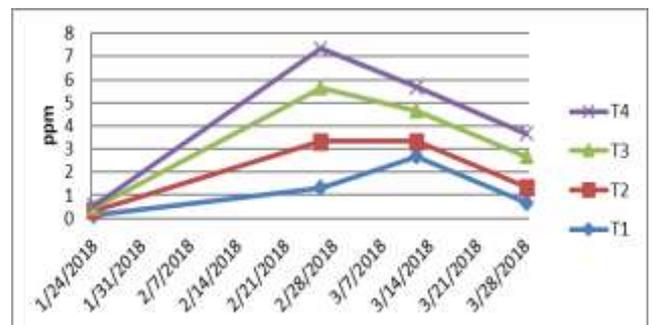


Fig. 4: Fortnightly measurement of mean Nitrate (ppm) of different treatments during experimental period.

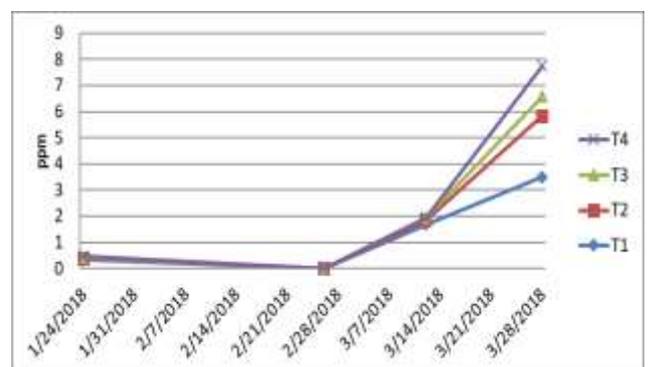


Fig. 5: Fortnightly measurement of mean Nitrite (ppm) of different treatments during experimental period.

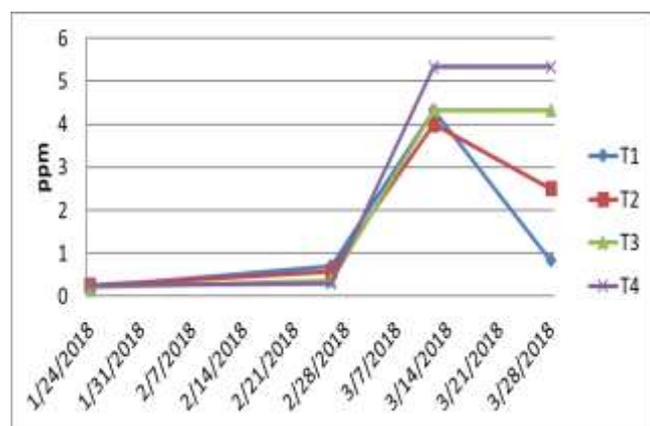


Fig. 6: Fortnightly measurement of mean Ammonia (ppm) of different treatments during experimental period

Growth Analysis

Table 2 shows that the stocking and harvest weight (gm) of catfish (*Clarius batrachus*) during the experimental period. There was significant difference in the stocking weight of fishes among all different treatments (Tab.2). In case of Av. Individual stock wt there was significant difference in T1 with T2, T2 with T3, T3 with T4. But there was no significant difference in T2 with T4. In case of Total harvest

weight there was significant difference in T1 with T2. Similarly, there was significant difference in T2 with T3 and in T3 with T4. However, there was no significant difference in T2 with T4. In case of average individual harvest weight of fish there was significant difference in T1 with T2, T2 with T3 and T3 with T4. But there was no significant difference in T4 with T2 and T2 with T4.

Hence, Table 3 shows the Feed Conversion Ratio (FCR), Average Daily Growth Rate (ADGR), Specific Growth Rate (SGR) of catfish during the experimental period. In case of FCR there was significant difference among all the treatments. Similarly, in case of ADGR there was also significant difference among all the treatments during the experimental period. Furthermore, in case of SGR there was also significant difference among all the treatments.

Table 4 shows that the feeding Rate per Fish Biomass (gm), Percentage Weight gain of catfish during the experimental period. In case of feeding rate per unit of biomass there was significant difference among all the treatments during the experimental period. Similarly, in case of weight gain percent there was also significant difference among all the treatments during the experimental period.

Table 2: Shows the stocking and harvest weight (gm) of catfish during the experimental period. (Mean \pm SE)

Parameters	Treatment			
	T1	T2	T3	T4
Total stocking no	14	14	14	14
Total stocking wt. (gm)	23.89 \pm 0.48 ^b	25.94 \pm 1.63 ^b	23.56 \pm 1.4 ^b	22.49 \pm 1.62 ^b
Average individual Stocking wt. (gm)	1.70 \pm 0.03 ^b	1.85 \pm 0.11 ^{ab}	1.68 \pm 0.10 ^b	1.60 \pm 0.07 ^{ab}
Total Harvest no	13	13	13	13
Total Harvest wt. (gm)	35.7 \pm 0.79 ^b	39.66 \pm 4.29 ^{ab}	35.60 \pm 2.30 ^b	32.04 \pm 0.83 ^{ab}
Average individual Harvest wt. (gm)	2.67 \pm 0.077 ^b	2.99 \pm 0.37 ^{ab}	2.66 \pm 0.50 ^b	2.40 \pm 0.91 ^{ab}

Different superscript letters within the same rows indicate significantly different at $p < 0.05$

Table 3: Feed Conversion Ratio (FCR), Average Daily Growth Rate (ADGR), Specific Growth Rate (SGR) of catfish during the experimental period. (Mean \pm SE)

Parameters	Treatment			
	T1	T2	T3	T4
FCR	0.32 \pm 0.006 ^b	0.31 \pm 0.007 ^b	0.32 \pm 0.003 ^b	0.33 \pm 0.012 ^b
ADGR	0.88 \pm 0.081 ^b	1.03 \pm 0.219 ^b	0.89 \pm 0.069	0.72 \pm 0.121 ^b
SGR	19.68 \pm 1.64 ^b	22.86 \pm 4.45 ^b	20.06 \pm 2.47 ^b	15.91 \pm 2.35 ^b

Different superscript letters within the same rows indicate significantly different at $p < 0.05$

Table 4: Feeding Rate per Fish Biomass (gm), Percentage Weight gain of catfish during the experimental period (Mean \pm SE)

Parameters	Treatment			
	T1	T2	T3	T4
Feeding rate per unit biomass	0.0283 \pm 0.0007 ^b	0.0317 \pm 0.0034 ^b	0.0287 \pm 0.0018 ^b	0.0257 \pm 0.0003 ^b
Weight gain %	49.55 \pm 4.90 ^b	52.03 \pm 6.97 ^b	50.75 \pm 4.69 ^b	43.10 \pm 7.76 ^b

Different superscript letters within the same rows indicate significantly different at $p < 0.05$

Discussion

The pH value between 8.1 to 8.4 seems fairly relevant during the experimental period. But dissolved oxygen level at the end of research period with value 5.4 mg/l in T3 on 27th March seems low in comparison to the previous outputs whereas DO level runs into good track from the middle of 6th March to 13th March. The temperature is found optimum ranging from 19.6°C to 24.1°C during the experimental period. During the experimental period, nitrite and nitrate seems optimum but ammonia gets relatively high with the value 5.33 ppm in T1 on 28th March, 2018. The level of ammonia from 24th Jan. to 28th Feb. seems optimum with the necessity of optimum level of water quality standard. After then it seems fluctuating up to 28th of Mar. 2018. There was significant difference in the total stocking weight and average individual stocking weight of fishes among all the treatment. In case of Total harvest weight there was significant difference in T1 with T2. Similarly, there was significant difference in T2 with T3 and in T3 with T4. The mean harvest weight of T2 (Feed mixed with probiotics/Microguard) seems fairly high in comparison to the other treatments during the experimental period. Rahman *et al.* 2013 had revealed that on the basis of poultry within 35 days (21st day of study) and was observed that the body weight in group D having Probiotics/ Microguard has highest body weight over other treatments. Fuller (1989) had reported that probiotics are feed additives that contain live microorganisms and promote beneficial effects on the host of favoring the balance of the intestinal microbes. It is also stated by Islam *et al.* (2004) that reveals the probiotics include live bacteria, yeast, their metabolites and pH adjusters, which contribute to maintain balance in intestinal micro flora. According to Choct *et al.* (1995), most of the feed ingredients contain some anti-nutritional factors and non-digested part which inhibit feed Utilization accompanied by poor growth. This adverse effect can be overcome by supplementation of exogenous carbohydrase (xylanase) enzymes improve digestibility of starch, protein, fat and apparent metabolisable energy in broiler feed. In

case of FCR, ADGR and SGR, there was significant difference among all the treatments. Similarly, In case of feeding rate per unit of biomass and % weight gain, there was significant difference among all the treatments during the experimental period.

Conclusion

Under laboratory conditions of IAAS, Paklihawa the growth and feed utilization of Catfish was observed. From the experimental trial, Probiotics i.e. Microguard mixed with supplementary feed gave the best result in terms of total weight of fish among other probiotics. Hence it is advisable to apply these kinds of non-toxic, environmentally friendly and profitable feed additives to get success in aquaculture sector.

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