

Project ID 497

Competitive Research Grant

Sub-Project Completion Report

on

**Evaluation of the Suitability and Efficacy of Potato
and Wheat as Prebiotic Compounds on the Growth
Performance, Survivability and Tissue Composition
of *Labeo rohita* and *Catla catla***

Project Duration

May 2017 to September 2018

**Department of Aquaculture
Bangladesh Agricultural University, Mymensingh-2202**



Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215



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Citation

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Project Implementation Unit

National Agricultural Technology Program-Phase II Project (NATP-2)

Bangladesh Agricultural Research Council (BARC)

New Airport Road, Farmgate, Dhaka – 1215

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Acronyms

BARC	:	Bangladesh Agricultural Research Council
BAU	:	Bangladesh Agricultural University
°C	:	Degree Celsius
cm	:	Centimeter
CRG	:	Competitive research grant
NATP	:	National Agriculture Technology Project
DoF	:	Department of Fisheries
et al.	:	Associates
FAO	:	Food and Agriculture Organization
FCE	:	Food conversion efficiency
FCR	:	Food conversion ratio
g	:	Gram
GDP	:	Gross Domestic Product
Kg	:	Kilogram
MS	:	Microsoft
PER	:	Protein efficiency ratio
PG	:	Pituitary gland
PIU	:	Project Implementation Unit
SGR	:	Specific Growth rate
SPSS	:	Statistical Package for Social Science
USAID	:	United States Agency for International Development
wt	:	Weight

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Executive Summary

Potato and wheat were assumed to have prebiotics properties i.e., non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon. Four diets were formulated, incorporating potato and wheat at different inclusion levels for *Labeo rohita* and *Catla catla* to assess the suitability and efficacy of potato and wheat as prebiotics which were not tested earlier.

The results of the present study demonstrated that prebiotic treated *L. rohita* attained high level growth in comparison with none treated ones. The highest survival rate, feed efficiency and growth performance were obtained at 15% potato supplementation (T₄) in feed. Another study proved significant role of wheat flour supplementation on the growth performance of *L. rohita* and the optimum inclusion level was 15%. Replacing 15% rice bran with 15% whole wheat flour was found quite effective because of its nutritional content and path of digestion. So it can be concluded that 15% whole wheat flour has paramount importance in enhancing the production of *L. rohita* and could be recommended to incorporate with feed.

Growth performance of *C. catla* on the basis of growth parameters such as mean weight gain, % weight gain and specific growth rate (SGR) and survival rate (%) were significantly higher in T₄ with 15% potato supplementation compared to other treatments containing 10% potato, 5% potato and 0% potato levels. In case of wheat supplementation in diet, the highest growth performance of *C. catla* was achieved at 10% inclusion level. So it can be concluded that inclusion of 10% whole wheat flour as prebiotic compound has maximum efficacy in enhancing the production of *C. catla* and recommended to incorporate with feed.

From the present investigations, it is revealed that the use of potato and wheat grain have accelerated the growth performances of *Catla catla* and *Labeo rohita* by increasing beneficial bacteria in the gut of these fishes. So initially, it may be concluded that the potato and wheat grain may be used as prebiotic to boost up the beneficial bacteria though much more research need to be performed. So, prebiotic can be used as alternatives to growth promoters but their combination strategy can be used to achieve good health and growth performance.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project:** Evaluation of the Suitability and Efficacy of Potato and Wheat as Prebiotic Compounds on the Growth Performance, Survivability and Tissue Composition of *Labeo rohita* and *Catla catla*

2. **Implementing organization:**

National Agricultural Technology Program-Phase II Project (NATP-2)

Bangladesh Agricultural Research Council (BARC)

New Airport Road, Farmgate, Dhaka – 1215

3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

Principal Investigator:

Name: Title: **Prof** First Name: **Md. Sazzad** Last Name: **Hossain**

Office Address: Professor, Dept. of Aquaculture, BAU, Mymensingh-2202, Bangladesh

Phone: +8801712776746

Email: sazzadbau@gmail.com

Co- Principal Investigator:

Name: Title: **Miss** First Name: **Zannatul** Last Name: **Ferdous**

Office Address: Assistant Professor, Dept. of Aquaculture, BAU, Mymensingh-2202, Bangladesh

Phone: +8801718324759

Email: zferdous58@yahoo.com

4. **Sub-project budget (Tk):**

4.1 Total: **37,96,113/-**

4.2 Revised (if any): **Not Applicable**

5. **Duration of the sub-project:**

5.1 Start date (based on LoA signed): 08 May, 2017

5.2 End date: 30 September 2018

6. **Justification of undertaking the sub-project:**

Aquaculture plays a vital role in many countries by offering better nutrition, higher income, earning foreign exchange and better employment opportunities. According to FAO (2018), aquaculture is the fastest growing sector of the world's animal production with an annual increase of about 5.8% since 2010. To sustain such high rates of fish production, a matching increase in fish feed production is imperative.

Among the farmed species *Labeo rohita* and *Catla catla* are the most important and popular fish species considering its taste and market demand. Many works have been done on feed formulation (Hossain and Hasan, 2000; Habib *et al.*, 2001; Dewan *et al.*, 2004) but high cost and fluctuating quality as well as the uncertain availability of fish meal have led to the need to identify alternative growth booster for fish feed formulation. To minimize the feed cost and to accelerate digestion, incorporation of prebiotics in fish feed is highly significant.

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon” (Gibson and Roberfroid, 1995). Gibson *et al.* (2004) elaborated the prebiotics concept by certain criteria viz. resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; fermentation by intestinal microflora and selective stimulation of the growth, and/or activity of intestinal bacteria associated with health and wellbeing. Stowell (2007) reviewed the existing prebiotics and classified them based on a set of common criteria. Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose and polydextose are recognized as the established prebiotics, whereas isomaltooligosaccharides (IMO), xylooligosaccharides (XOS), and lactitol are categorized as emerging prebiotics. Chicory root inulin-derived (FOS), wheat bran-derived arabinoxyloligosaccharides (AXOS) and xylooligosaccharides (XOS) proved to have huge applications (Sabater-Molina *et al.*, 2009; Femia *et al.*, 2010; Xu *et al.*, 2009). Mannitol, maltodextrin, raffinose, lactulose, and sorbitol are also prebiotics with proven health properties (Yeo and Liong, 2010; Vamanu and Vamanu, 2010; Mandal *et al.*, 2009). Whole grains are loaded with prebiotics which are indigestible fibers that stimulate the growth of the good bacteria within your microbiome. These are not absorbed in small intestine of healthy individuals but later are fermented by natural microflora of the colon to produce short-chain fatty acids (SCHFA) (Vaidya and Sheth, 2010).

Prebiotic compounds are mostly plant-derived additives containing carbohydrates and also have been associated with several health promoting effects (Oyetayo & Oyetayo, 2005). The use of traditional means of combating disease such as the use of antibiotic for fish is costly and some farmers could not afford them. As a result, prebiotic is preferred as one of the health promoting compounds in developing dietary supplementation strategies in diet preparation compared with antibiotics. However, little attention has been given to prebiotics in aquaculture; the objective of this study is to evaluate potato and wheat supplementation as a prebiotic compound into the diets for *Labeo rohita* and *Catla catla* and its effect on growth performances.

Therefore, in order to attain economically sustainable, environment friendly and viable production, research interest had been directed towards evaluating the use of prebiotic sources in formulating effective low-cost fish feed.

7. Sub-project goal:

Innovation of a cost effective fish feed by incorporating potato and wheat for boosting up fish production which would uplift nutrition, livelihood and income of fish farmers.

8. Sub-project objective (s):

The aim of the project is to evaluate the growth performances of *Labeo rohita* and *Catla catla* by feeding pellet feed supplemented with potato and wheat which would be helpful for the rural people in uplifting their nutrition, livelihood additional income. The specific objectives are:

- i) To determine the effect of potato and wheat as prebiotics on the growth performance of *Labeo rohita* and *Catla catla* and
- ii) To evaluate the impact of potato and wheat supplemented feed to increase the beneficial gut microflora of *Labeo rohita* and *Catla catla*.

9. Implementing location (s):

The present aquarium trials were conducted in the Wet Laboratory of Faculty of Fisheries but samples were analyzed in the Fish Nutrition Laboratory and Fish Disease Laboratory of the Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh.

10. Methodology:

10.1. Experimental site

The research was carried out in 24 glass aquaria (50L capacity) for two species such as *Labeo rohita* and *Catla catla* at the wet laboratory, adjacent to the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. Stored ground water in cemented tank was used for rearing the fish during the experimental period. Aeration was provided from a 1.0-HP compressor to all the experimental aquaria.

10.2 Collection of fish fry:

Fingerling of *Labeo rohita* and *Catla catla* were collected from a private hatchery in Mymensingh. Collected fingerlings were acclimated to laboratory condition in two big glass tanks (120 L capacity each) for 2 weeks by feeding with a commercial carp diet (Provita Feed).

10.3 Feed formulation:

The basal experimental diets were prepared using commonly available ingredients (Tables 1 and 2). The ingredients such as fish meal, rice bran, wheat bran, molasses, vitamin C, minerals and vitamin premix were purchased from local market. Four grade levels of potato and wheat grains at 0, 5%, 10%, and 15% were incorporated in the basal diet by replacing rice bran. The ingredients were grinded, milled, weighed, mixed and pelleted with meat mincer and passed through 0.8 mm diameter sieve (Plate 1). After cooling in room temperature, the pelleted feeds were air dried and put in air-tight containers (Plate 2). All diets were stored at -20°C until fed.



A



B

Plate 1: Grinder (A) and Hand pellet machine (B)



A



B

Plate 2: Drying of feed (A) and labeling feeds in pots (B)

Table 1. Formulation of the basal diet using potato (dry matter basis)

Ingredients (%)	Diet 1- Control	Diet 2	Diet 3	Diet 4
Fish meal	30	30	30	30
Rice bran	30	25	20	15
Potato	0	5	10	15
SBM	20	20	20	20
MOC	12	12	12	12
Molasses	5.5	5.5	5.5	5.5
Vitamin premix	1	1	1	1
Mineral premix	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5

Table 2. Formulation of the basal diet using wheat flour (dry matter basis)

Ingredients	Diet 1- Control	Diet 2	Diet 3	Diet 4
Fish meal	30	30	30	30
Rice bran	30	25	20	15
Wheat flour	0	5	10	15
SBM	20	20	20	20
MOC	12	12	12	12
Molasses	5.5	5.5	5.5	5.5
Vitamin premix	1	1	1	1
Mineral premix	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5

10.4 Feeding of fingerlings and data collection:

Fifteen fish were stocked randomly into triplicate aquariums for each dietary group with nearly uniform biomass. All experimental fish were acclimated to the basal diet (without prebiotic compound) for 2 weeks prior to start of the growth trial. All groups of fish were fed their respective diet at 10% of body weight initially and gradually reduced to maintain a level close to apparent satiation. Any uneaten food was collected 1 h after every feeding and the dry matter contents were determined for both supplied and uneaten food (Plate 3, 4). The daily ration was divided into two equal parts and fed at 9:30 am and 5:00 pm. After each biweekly weighing, ration sizes were adjusted according to their body weights for the next period of feeding. A triplicate feeding trial was conducted over a 70 days period. At the end of the experiment, the fish were measured for growth performance and proximate analysis (AOAC, 2000). Water quality parameters such as dissolved oxygen, pH, nitrate and ammonia were monitored biweekly.



A



B

Plate 3: Siphoning (A) and water supplying in aquaria (B)



A

B

Plate 4: Weighing of feed (A) and feeding (B)

10.5 Analytical method

10.5.1 Moisture

Moisture content was determined in triplicate by placing an accurately weighed amount (about 2-3 g ground sample in a pre-weighed porcelain crucible in a hot air oven (Gallenkamp, HOTBOX, Model OVB-305) at 105°C for 24hr until a constant weight was obtained. The loss of weight was calculated as percent moisture content.

$$\text{Moisture (\%)} = \frac{\text{Original sample weight (g)} - \text{Dried sample weight (g)}}{\text{Original sample weight (g)}} \times 100$$

10.5.2 Ash

Ash content of the samples was determined by burning about 2g sample in a Muffle Furnace (Philip Harris Ltd, England), for 6 hours at a temperature of 550°C. After cooling, the crucible was weighed again. The ash content was calculated and expressed as percentage of the original sample. Ash content was determined by using the following formula.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

10.5.3 Crude protein

Crude protein was determined indirectly by measuring total nitrogen content by standard Kjeldahl method. Known quantities of sample (0.5g), catalyst mixture (1.1g) and concentrated H₂SO₄ (10ml) were taken in a Kjeldahl flask and was digested in digestion unit (Digestor, Model-2020) for 45 minutes to obtain a clear solution. The obtained solution was then distilled in distillation unit (Kjeldahl system, Distilling unit, Model-1026) using 33% sodium thiosulphate (Na₂S₂O₃), 40% sodium hydroxide (NaOH) and 40% boric acid solution and was titrated with standard hydrochloric acid (HCl). The percentage of total nitrogen was multiplied by the empirical factor of 6.25 or 5.87 assuming that protein contains.

$$\text{Nitrogen (\%)} = \frac{\text{Milli equivalent of nitrogen (0.014)} \times \text{titrant value (ml)} \times \text{strength of HCl}}{\text{Sample weight (g)}} \times 100$$

Crude protein (%) = 6.25 × % Nitrogen, for animal source.

Crude protein (%) = 5.87 × % Nitrogen, for plant source.

10.5.4 Crude lipid

Crude lipid content was determined by extracting the weighed sample in acetone for 6 hours in a Soxhlet apparatus. The collected oil was transferred in a small pre-weighed beaker and kept in oven for 20 minutes to evaporate the acetone. Oil in the beaker was weighed by an electric balance and the percentage of total lipid was calculated using the following formula:

$$\text{Crude lipid (\%)} = (\text{Extracted lipid /sample weight}) \times 100$$

10.5.5 Crude fibre

A small amount of finely ground sample (0.5g) was taken into a filter crucible, and was inserted into the hot extraction unit (Hot extractor, Model 1017). About 150 ml of preheated 0.128 M H₂SO₄ was added into the reagent heating cylinder and 2-3 drops of N-octanol, an anti-foaming agent was added and digested for 30 minutes. Acid was then removed by vacuum filtering followed by washing with boiling water. The residue in the flask was boiled with required amount of 0.223 M KOH for 30 minutes and filtered with subsequent washing in boiling water and acetone. Washed samples were taken out from extractor along with filter and washed with 30 ml acetone. The residual samples were air dried at 105°C overnight and ignited in muffle furnace at 550°C for 6 hours. The loss in weight represented the crude fibre.

$$\text{Crude fibre (\%)} = \frac{\text{Wt. of sample after air drying (g)} - \text{Wt. of sample after ashing (g)}}{\text{Sample weight (g)}} \times 100$$

10.6 Growth performance

10.6.1 Weight gain (g)

Weight gained refers to as the difference between final weight and initial weight.

The formula:

$$\text{Weight gain (g)} = \text{mean final weight (g)} - \text{mean initial weight (g)}$$

10.6.2 Percent weight gain (%)

Percent weight gain was calculated by using the following formula:

$$\text{Percent weight gain (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Where, W₁ = the mean initial fish weight, W₂ = the mean final fish weight.

10.6.3 Specific growth rate (% /day)

The specific growth rate in the immediate change in weight of fish calculated as the percentage increase in body weight per day over given time interval. Growth in terms of weight was calculated by subtracting the initial weight of fish (at the time of release) from final weight of the same after. The specific growth rate (SGR) was determined by following formula:

$$\text{Specific growth rate (\%/day)} = \frac{\text{Ln } W_2 - \text{Ln } W_1}{T_2 - T_1} \times 100$$

Where,

W₁ = Initial live body weight (g) at time T₁

W₂ = Final live body weight (g) at time T₂

T₂-T₁ = No. of days of the experiment

10.6.4 Feed Conversion Ratio

Feed conversion ratio (FCR), in its simplest form a comparison of the amount of feed used per unit weight gain of the species being grown, offers a measure of aquaculture production efficiency. FCR was determined by the following formula:

$$\text{FCR} = \frac{\text{Feed fed (dry weight)}}{\text{Live weight gain (g)}}$$

10.6.5 Feed Conversion Efficiency

The feed conversion efficiency (FCE) of a feed is normally measured by the amount necessary to produce a unit weight of fish. FCE was determined by the following formula:

$$\text{FCE} = \frac{\text{Live weight gain (g)}}{\text{Feed fed (dry weight)}}$$

10.6.6 Protein Efficiency Ratio

Protein efficiency ratio (PER) is based on the weighed gain of a test subject divided by its intake of a particular food protein during the test period. From 1919 until very recently, the PER had been a widely used method for evaluating the quality of protein in food. PER was determined by using the following formula:

$$\text{PER} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

10.6.7 Nitrogen free extracts (NFE)

Nitrogen free extracts was calculated by subtracting the sum of the percentage contents of moisture, crude protein, lipid, ash, and crude fibre from 100 (Castell and Tiews, 1980). It is a soluble carbohydrate.

$$\text{NFE (\%)} = \{100 - (\text{moisture} + \text{crude protein} + \text{crude lipid} + \text{ash} + \text{crude fibre})\}$$

10.6.8 Survival rate (%)

In each treatment survival rate of fry was estimated on the basis of number of fish remained at the end of the experiment in relation to the number stocked. Survival rate of fish was calculate by counting the actual number of fish survived divided by initial number of stocked fish and multiplying by 100. So the equation of survival rate is:

$$\text{Survival rate (\%)} = \frac{\text{Total no of fish harvested}}{\text{Total number of stocked}} \times 100$$

10.7 Water quality parameters

The water quality parameters such as water temperature, dissolved oxygen (DO) and pH were monitored weekly throughout the experimental period.

10.7.1 Temperature

Water temperature (°C) of the aquaria was measured weekly with the help of a Celsius thermometer (TL8009A).

10.7.2 Dissolved oxygen

Dissolved oxygen (mg/L) of the water was measured weekly by using an oxygen meter (Oxymeter WTW, Multi 340i).

10.7.3 pH

Electronic pH meter (Jenway, model 3020 UK) was used to measure the pH of water in every seven days.

10.8 Isolation and enumeration of microbiota from experimental fish

Bacterial load of gut in fishes were analyzed by consecutive decimal dilution method in Disease Laboratory of Bangladesh Agricultural University. In this method following procedure were followed:

1. Preparation of equipments:

Conical flasks, petri-dishes, L-shaped rods and other necessary things were washed with detergent, dried at 70°C in oven and then sterilized at 170°C for 1 hour in hot air oven.

2. Media Preparation:

Trypton Soya Agar (TSA) was mixed with distilled water at a rate of 40 g/L of water, heated in hot plate for proper mixing and sterilized in autoclave at 121°C for 15 minutes. After cooling to 60°C the sterilized media was poured to petridishes at a rate of 30 mL per dish. After cooling and solidification, the petridishes were kept upside down at 4°C for further use.

3. Preparation of Sample:

Fish were killed and 1 g gut collected from the freshly killed fish, homogenized with 10 mL physiological saline (0.87% NaCl solution) in a glass homogenizer. For consecutive decimal dilution physiological saline (PS) was used as diluents. From the stock solution 1 mL of stock was diluted with 9 ml of PS to make a dilution of 10 fold (dilution factor- 1). Following the same procedure the stock solution was diluted for n times.

4. Inoculation and Incubation:

Diluted 1 ml sample is spreaded onto the TSA culture medium with L-shaped rod after inoculating with sterilized syringe. Culture was duplicated for each of the stock solution to prevent any error that might be caused. Plates were incubated in temperature regulated incubator at 37°C for 24 hours.

5. Colony counting:

After incubation, bacterial colonies were counted with a digital colony counter for each plate.

6. Bacterial load estimation:

After counting colony in each plate, bacterial load was calculated according to Mamnur *rashid et al.*, (1994):

Bacterial load (CFU/g of gut) = Nos. of colony in each plate* 10ⁿ*100

(CFU= Colony Forming Unit; n= dilution factor)

10.9 Data analysis:

Statistical analysis of the data was performed by ANOVA. Duncan's New Multiple Range Test (Duncan, 1955) was applied to test the significance of variation between the treatment means. Standard error (SE) of the treatment means was calculated from the residual mean square in the analysis of variance. All statistical analyses were carried out by MS EXCEL 2000 (version 7.0).

11. Results and discussion:

11.1 Suitability and efficacy of potato as prebiotic compound on the growth performance of Rohu, *Labeo rohita*.

The value of water temperature, dissolved oxygen, pH, ammonia and alkalinity were found in the suitable range in different treatments. The highest weight gain of Rohu was found 2.73 ± 0.40 g in T₄

(with 15% Potato) after 63 days of experiments. Weight gain (g), % weight gain and SGR (% per day) varied from 1.29 ± 0.25 to 2.73 ± 0.40 ; 74.32 ± 14.17 to 157.01 ± 22.77 and 0.38 ± 0.06 to 0.65 ± 0.06 , respectively. Survival rates were $97.78 \pm 2.22\%$, 100% , 100% and 100% in T_1 , T_2 , T_3 and T_4 respectively. Values of FCR, FCE and PER were varied from 2.12 ± 0.22 to 3.49 ± 0.47 ; 0.29 ± 0.04 to 0.48 ± 0.05 , and 0.68 ± 0.09 to 1.09 ± 0.11 , respectively. The highest FCR (3.49 ± 0.47) was found in T_1 and lowest FCR (2.12 ± 0.22) was found in T_2 . The highest PER (1.09 ± 0.11) was found in T_2 and lowest PER (0.68 ± 0.09) was found in T_1 . Growth of Rohu was found higher when the highest prebiotic (15% Potato) diet was fed to experimental fish. The present study highlighted the effects of dietary prebiotics (potato) on different growth parameters of Rohu.

11.1.2 Physicochemical parameters of the aquarium water

The water quality parameters were recorded weekly throughout the experimental period. Temperature ($^{\circ}\text{C}$), dissolved oxygen (mg/L), pH, alkalinity (ppm) and ammonia (mg/L) were ranged 28.95 to 30.61, 7.06 to 7.36, 7.25 to 7.59, 198.59 to 201.41, 0.18 to 0.24 respectively (Table 3).

Table 3: Mean water quality parameters in different treatments during the study period

Water quality parameters	Value range
Temperature ($^{\circ}\text{C}$)	29.78 ± 0.83
Dissolved oxygen (mg/l)	7.21 ± 0.15
pH	7.42 ± 0.17
Alkalinity (ppm)	200 ± 1.41
Ammonia (mg/l)	0.21 ± 0.03

11.1.3 Growth performance of *L. rohita*

Growth performance of *L. rohita* in terms of final weight (g) gain, weight (g) gain, percent weight (g) gain, specific growth rate (SGR% per day) and survival rate under different treatments for a period of 63 days is presented in Table 4.

Table 4: Growth performances of *L. rohita* fry fed with potato enriched feed at different treatments during the study period

Variable parameters	T_1 (0% Potato)	T_2 (5% Potato)	T_3 (10% Potato)	T_4 (15% Potato)
Initial weight (g)	1.74 ± 00	1.74 ± 00	1.74 ± 00	1.74 ± 00
Final weight (g)	3.03 ± 0.25	4.33 ± 0.31	3.70 ± 0.23	4.47 ± 0.40
Weight gain (g)	$1.29^a \pm 0.25$	$2.59^b \pm 0.31$	$1.96^a \pm 0.23$	$2.73^b \pm 0.40$
% Weight gain	$74.32^a \pm 14.17$	$149^b \pm 17.55$	$112.45^a \pm 13.23$	$157.01^b \pm 22.77$
SGR (%/day)	$0.38^a \pm 0.06$	$0.63^b \pm 0.05$	$0.52^a \pm 0.05$	$0.65^b \pm 0.06$
FCR	$3.49^a \pm 0.47$	$2.12^b \pm 0.22$	$2.61^b \pm 0.20$	$2.15^b \pm 0.15$
FCE	$0.29^a \pm 0.04$	$0.48^b \pm 0.05$	$0.38^a \pm 0.03$	$0.47^b \pm 0.04$
PER	$0.68^a \pm 0.09$	$1.09^b \pm 0.11$	$0.87^a \pm 0.07$	$1.06^b \pm 0.07$
Survival rate (%)	$97.78^a \pm 2.22$	$100^b \pm 00$	$100^b \pm 00$	$100^b \pm 00$

Values are means \pm SD. Different letters show significant differences among different treatments ($p < 0.05$).

The mean difference is significant at the 0.05 level.

11.1.3.1 Weight gain (g)

There was no significant ($p < 0.05$) difference in initial weight 1.74 ± 0.00 g of Rohu, *L. rohita* among four treatments. The mean weight gains of Rohu at the end of the experiment were 1.29 ± 0.25 ; 2.59 ± 0.31 ; 1.96 ± 0.23 and 2.73 ± 0.40 g in T₁, T₂, T₃ and T₄, respectively with significant difference among treatments. The highest mean weight gain 2.73 ± 0.40 g was attained in T₄ where 15% potato was incorporated in fish feed (Table 4 and Figure 1).

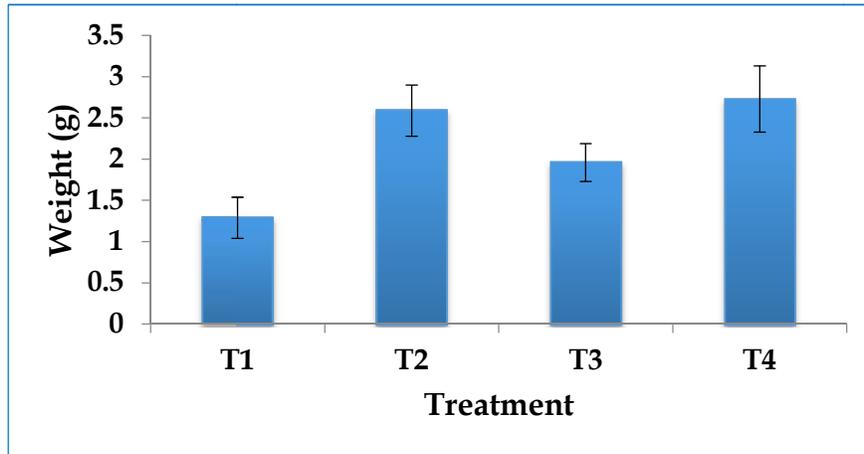


Figure 1: Mean body weight gain (g) of *L. rohita* fed potato enriched diets in different treatments during the experimental period.

11.1.3.2 Percent weight gain

The mean percent weight gains of *L. rohita* fry were found $74.32\% \pm 14.17$, $149.04\% \pm 17.55$, $112.45\% \pm 13.23$ and $157.01\% \pm 22.77$ in T₁, T₂, T₃ and T₄, respectively (Table 4). The significantly highest and lowest mean percent weight gains were recorded $157.01\% \pm 22.77$ and $74.32\% \pm 14.17$ in T₄ (15% potato) and T₁ (0% potato) (Figure 2).

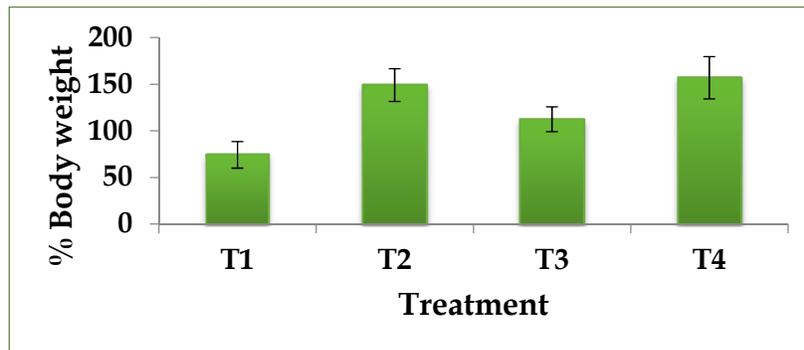


Figure 2: Mean percentage of body weight gain of *L. rohita* fed potato enriched diets in different treatments during the experimental period.

11.1.3.3 Specific Growth Rate (SGR) of *L. rohita*.

The mean specific growth rates (%/day) of *L. rohita* fry obtained at the end of the trials were 0.38 ± 0.06 , 0.63 ± 0.05 , 0.52 ± 0.05 and 0.65 ± 0.06 in T₁, T₂, T₃, T₄, respectively with significant difference among treatments. The highest mean SGR value was recorded 0.65 ± 0.06 in T₄ (Table 4 and Figure 3).

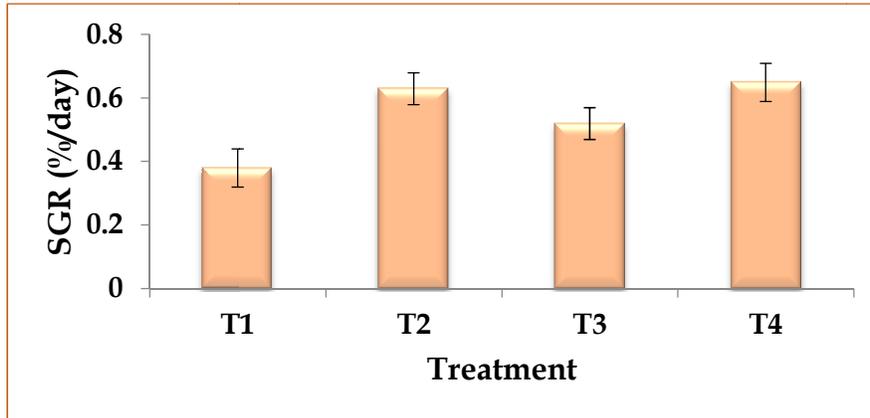


Figure 3: Mean Specific growth rate (%/day) of *L. rohita* fed potato enriched diets during the experimental period.

11.1.3.4 Food conversion ratio (FCR)

FCR in different treatments ranged from 2.12 ± 0.22 to 3.49 ± 0.47 (Table 4). The highest FCR was obtained in T₁ and the lowest in T₂ (Figure 4).

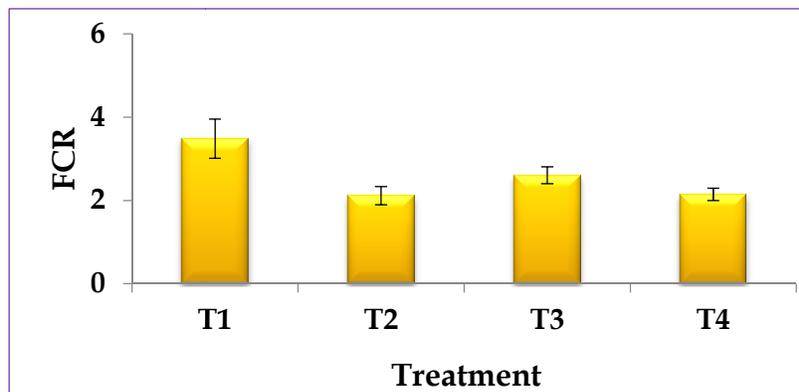


Figure 4: Food conversion ratio (FCR) of *L. rohita* fed potato enriched diets in different treatments during the experimental period.

11.1.3.5 Food conversion efficiency (FCE)

FCE in different treatments ranged from 0.29 ± 0.04 to 0.48 ± 0.05 (Table 4). The highest FCE was obtained in T₂ and lowest FCE was obtained in T₁ (Figure 5).

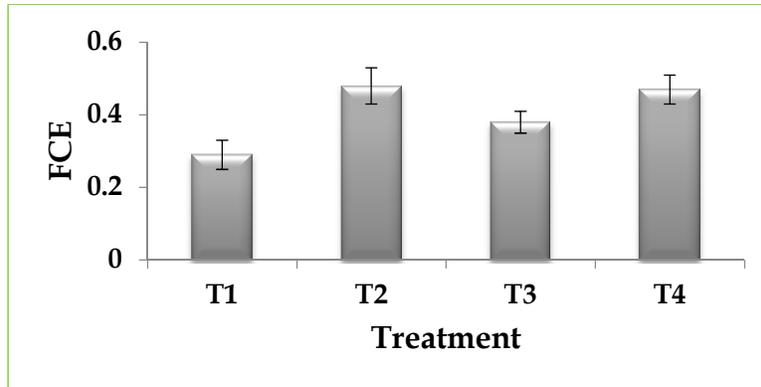


Figure 5: Food conversion efficiency (FCE) of *L. rohita* fed potato enriched diets in different treatments during the experimental period.

11.1.3.6 Protein efficiency ratio (PER)

PER in different treatments varied from 0.68 ± 0.09 to 1.09 ± 0.11 (Table 4). The significantly highest PER was found in T₂ and lowest was in T₁ (Figure 6).

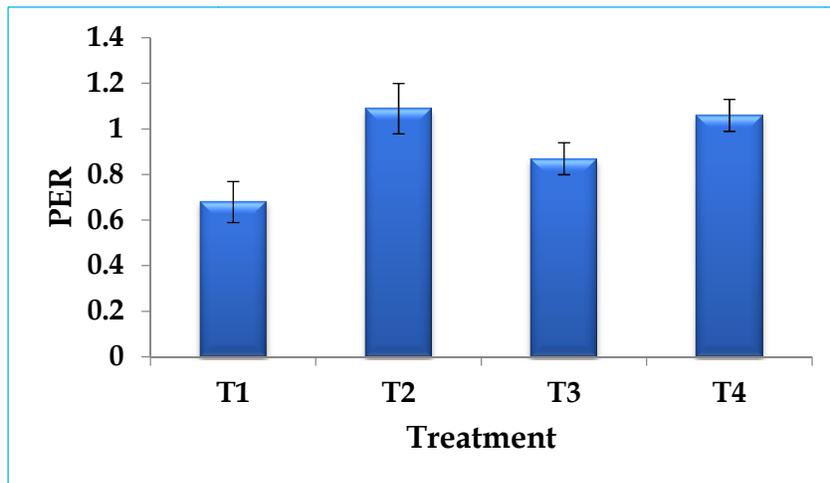


Figure 6: Protein efficiency ratio (PER) of *L. rohita* given fed potato enriched diets in different treatments during the experimental period.

11.1.3.7 Survival rate

Survival rate is an important indicator for fish production. The survival rate at T₁ was found 97.78% ± 22, But 100% survival rate was observed in prebiotic treated group T₂, T₃ and T₄ (Figure 7).

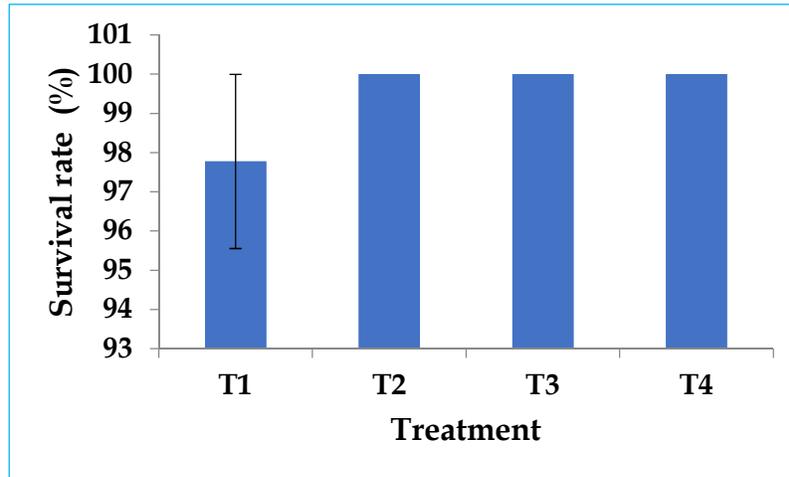


Figure 7: Survival rate of *L. rohita* fed potato enriched diets in different treatments during the experimental period.

11.1.1 Suitability and efficacy of potato as prebiotic compounds on the growth performance and survival rate of Catla (*Catla catla*)

Twelve fingerlings, initial individual fish weight of 7.5 ± 0.0 g, were released per aquarium. Experiment was carried out under 4 different treatments (T₁, T₂, T₃ and T₄) each with 3 replications were used having different amount of potato (Diet 1- 0% potato; Diet 2-5% potato; Diet 3-10% potato and Diet 4-15% potato). All four diets having a constant inclusion level of the following ingredients: fish meal 30%, rice bran 30%, molasses 5%, soybean oil 4% and vitamin and mineral premix 1%. Feed were supplied at 5% body weight twice daily in the morning at 9:00 am and in the afternoon at 5:00 pm throughout the study period. Sampling was done at 7 days interval throughout the experimental period by using digital measuring balance and data was recorded after sampling. Water quality parameters were maintained within the acceptable range during the experimental period. Final weight (g), weight gain (g), percent weight gain (%), SGR (%/day) and PER varied from 10.61 ± 0.43 to 13.28 ± 0.17 , 3.11 ± 0.43 to 5.78 ± 0.17 , 41.42 ± 5.67 to 77.11 ± 2.27 , 0.55 ± 0.03 to 0.91 ± 0.03 and 0.55 ± 0.005 to 0.90 ± 0.009 , respectively. Highest FCR (6.18 ± 0.10) was found in T₁ followed by T₂, T₃ and the lowest FCR (3.59 ± 0.18) was found in T₄. Highest PER (0.90 ± 0.009) was found in T₄ followed by T₃, T₂ and the lowest PER was found in T₁ which was (0.55 ± 0.005). The gut microbiota in catla fish in case of TSA agar media ranged from 1.7×10^6 CFU/ml to 9.6×10^7 CFU/ml and MRS agar media was 5.7×10^2 CFU/ml to 7.5×10^4 CFU/ml. There was no fish mortality observed during experimental period.

11.1.2.1 Growth performances of *Catla catla*

The growth performances of *C. catla* in terms of initial weight (g), Final weight (g), weight gain (g), percent weight gain (%) and specific growth rate (%/day) were calculated at the end of the experiment.

Table 5. Effect of different treatments on growth performance, feed utilization and survival of catla (*C. catla*) fed with potato enriched diet (with variable % of potato) during the study period.

Variable parameters	T ₁ (0% Potato)	T ₂ (5% Potato)	T ₃ (10% Potato)	T ₄ (15% Potato)	LSD	Level of sign
Initial weight (g)	7.5 ± 0.00	7.5 ± 0.00	7.5 ± 0.00	7.5 ± 0.00		ND
Final weight (g)	10.61 (± 0.43)c	12.29 (± 0.11)b	12.89 (± 0.17)a	13.28 (± 0.17)a	0.271	**
Weight gain (g)	3.11 (± 0.43)c	4.79 (± 0.11)b	5.39 (± 0.17)a	5.78 (± 0.17)a	0.271	**
% weight gain	41.42 (± 5.67)c	63.87 (± 1.47)b	71.91 (± 2.20)a	77.11 (± 2.27)a	3.616	**
SGR (%/day)	0.55 (± 0.03)c	0.78 (± 0.02)b	0.86 (± 0.00)a	0.91 (± 0.03)a	0.034	**
FCR	6.18 (± 0.10)a	4.07 (± 0.16)b	3.84 (± 0.18)b	3.59 (± 0.18)b	0.620	**
FCE	0.12 (± 0.02)b	0.17 (± 0.26)a	0.18 (± 0.01)a	0.20 (± 0.02)a	0.011	**
PER	0.55 (± 0.005)b	0.80 (± 0.002)a	0.84 (± 0.001)a	0.90 (± 0.009)a	0.011	**
Survival rate (%)	100 (± 0.00)c	100 (± 0.00)b	100 (± 0.00)a	100 (± 0.00)a	0.00	ND

Values given in bracket are standard deviation. The values in the same row having similar letter (s) do not differ significantly otherwise differ significantly ($p < 0.005$) as per Duncan Multiple Range Test (DMRT).

11.1.2.1.1 Initial weight (g)

The initial average weight of individual *C. catla* in different treatments was 7.5g (Table 5 and figure 8).

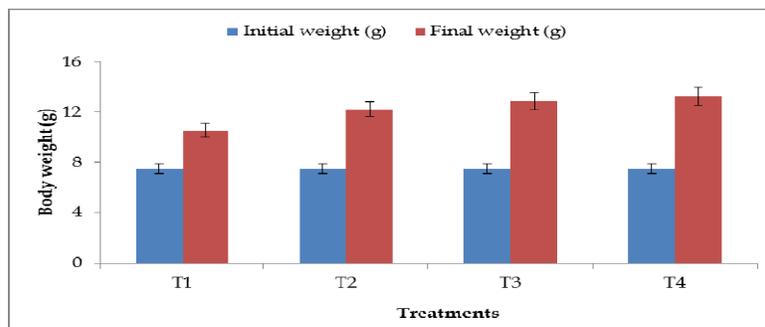


Figure 8. Mean final weight (g) of individual *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.2 Final weight (g)

The mean final weight of *C. catla* in different treatments varied from 10.61 g to 13.28 g. The mean weight gain (g) in treatment T₄ was found highest followed by T₃, T₂ and T₁, respectively (Table 5).

[

11.1.2.1.3 Weight gain (g)

The mean weight gain of individual *C. catla* in different treatments ranged from 3.11g to 5.78g. The mean weight gain of experimental fish was found highest in treatment T₄ followed by T₃, T₂ and T₁, respectively (Figure 9).

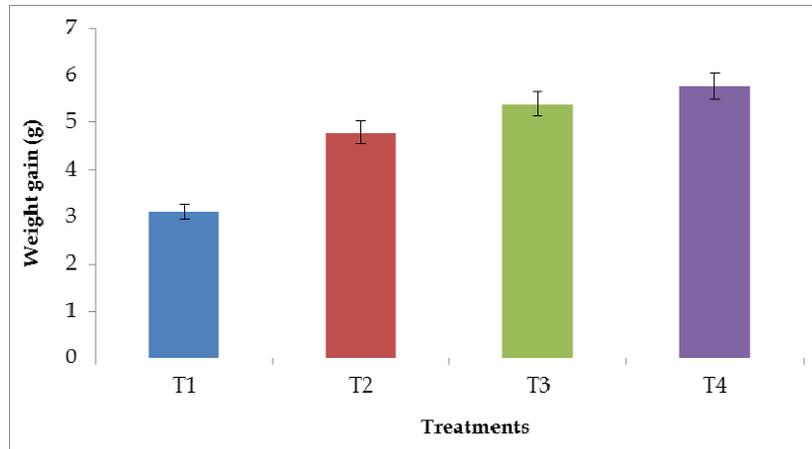


Figure 9. Mean weight gain (g) of *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.4 Percent weight gain (%)

The percent weight gain (%) of 12 fish in different treatments ranged from 41.42% to 77.11%. The highest percent weight gain (%) was obtained 77.11 (± 2.27)a in treatment T₄ followed by T₃, T₂ and T₁, respectively (Figure 10). There was significant ($p < 0.05$) variation in percent weight gain between T₁ and T₃; T₁ and T₄; T₂ and T₃; T₂ and T₄ but no significant variation was found between T₃ and T₄ (Table 5).

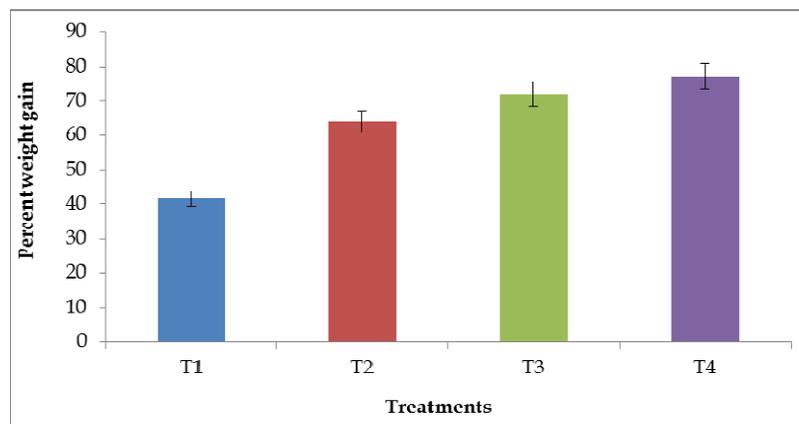


Figure 10. Mean percent weight gain (g) of *C. catla* fed potato enriched diets during the experimental period.

11.1.2.1.5 Specific growth rate (%/day)

The fishes were sampled at 7 days interval to measure the weight gain of fish in order to determine specific growth rate (SGR). The specific growth rate (%/day) ranged from 0.55% to 0.91% /day (Table 5). The highest specific growth rate (0.91%/day) was found in treatment T₄ followed by T₃, T₂ T₁, respectively. There was significant ($p < 0.05$) variation of Specific growth rate between T₁ and T₃; T₁ and T₄; T₂ and T₃; T₂ and T₄ but no significant variation found between T₃ and T₄ (Figure 11).

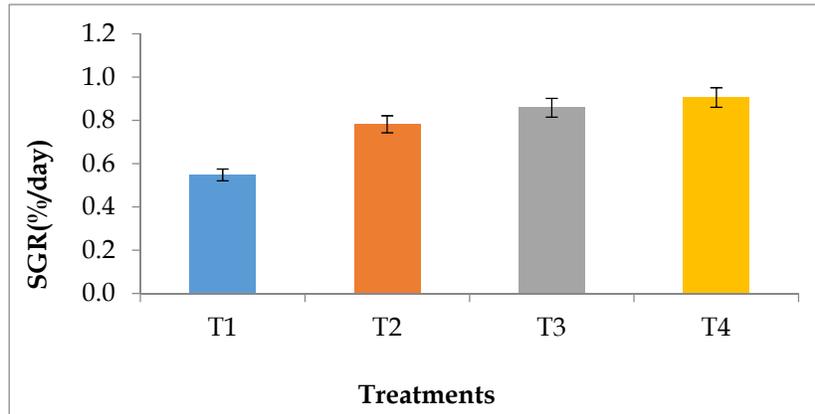


Figure 11. Mean Specific growth rate (%/day) of *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.6 Food conversion ratio (FCR):

Mean food conversion ratio (FCR) in different treatments ranged from 3.59 to 6.18 (Table 5). The highest FCR was obtained in treatment T₁ followed by T₄, T₃, and T₂, respectively. There was no significant ($p \geq 0.05$) variation in mean food conversion ratio (FCR) among T₂, T₃ and T₄ but has significant variation ($p < 0.05$) between T₁ and other treatments (Figure 12).

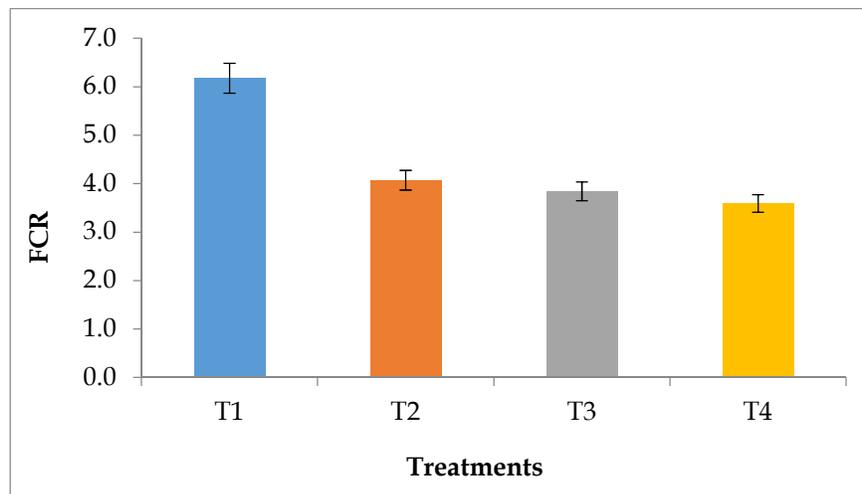


Figure 12. Mean Food conversion ratio of *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.7 Food conversion efficiency (FCE)

Mean food conversion efficiency (FCE) in different treatments ranged from 0.12 to 0.20 (Table 5). The highest FCE was obtained in treatment T₄ followed by T₃, and T₁, T₂, respectively. The highest FCE, 0.20 (± 0.02)_a was obtained in treatment T₄ followed by T₃, T₂, and T₁, respectively. There was no significant ($p \geq 0.05$) variation in mean food conversion ratio (FCR) among T₂, T₃ and T₄ but has significant variation ($p < 0.05$) between T₁ and other treatments (Figure 13).

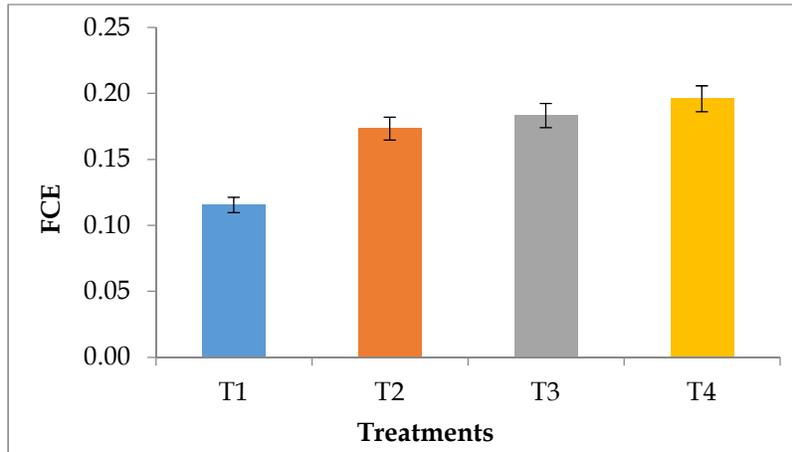


Figure 13. Mean Food conversion efficiency of *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.8 Protein efficiency ratio (PER)

Mean protein efficiency ratio (PER) in different treatments varied from 0.55 to 0.90 (Table 5). The highest protein efficiency ratio (PER) 0.90 (± 0.009)_a was found in treatment T₄ followed by T₃, T₂, and T₁, respectively. There was no significant ($p \geq 0.05$) variation in mean protein efficiency ratio (PER) among T₂, T₃ and T₄ but has significant variation ($p < 0.05$) between T₁ and other treatments (Figure 14).

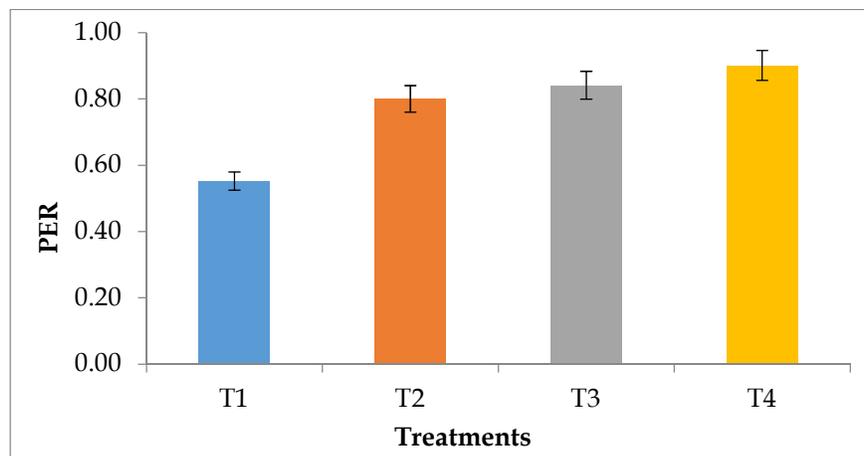


Figure 14. Mean Protein efficiency ratio of *C. catla* fed potato enriched diets in different treatments during the experimental period.

11.1.2.1.9 Survival rate (%)

The mean survival rate (%) of *C. catla* under different treatments was 100%. (Table 5 and Figure 15). There was no significant ($p \geq 0.05$) variation in survival rate of *C. catla* among four treatments.

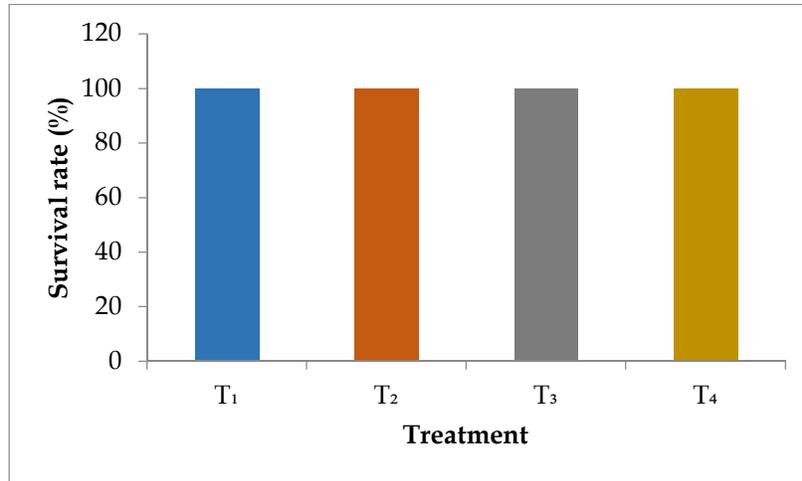


Figure 15. Mean Survival rate of *C. catla* fed by potato enriched feed in different treatments during the experimental period.

11.1.2.2 Gut microbiota in TSA and MRS agar media in different treatments are given below

In this experiment the bacterial colony in *C. catla* was measured for different treatments after rearing 63 days in aquarium. In case of TSA agar media the highest range of gut microbiota ($9.6 \times \text{CFU/ml}$) was found in T₄ that contain 15% potato followed by T₂, T₃ and T₁, respectively (Table 6) and in case of MRS agar media the highest range was found in T₂ ($7.5 \times \text{CFU/ml}$) which contain 5% potato followed by T₄, T₃ and T₁, respectively (Table 6). The lowest bacterial load was found in treatment 1 under controlled condition which contains 0% potato in diet composition.

Table 6. Plate count of *C. catla* gut microbiota in TSA and MRS agar media feed with potato as prebiotic compound after 63 days of rearing in aquaria.

Treatment	TSA agar media (CFU/ml)	MRS agar media (CFU/ml)
Treatment 1	1.7×	5.7×
Treatment 2	1.2×	7.5×
Treatment 3	1.1×	1.22×
Treatment 4	9.6×	3.03×

11.1.2.3 Water quality parameter

The water quality parameters such as temperature, dissolved oxygen and pH of different aquariums were measured throughout the experimental period. The range of temperature, dissolved oxygen and pH were 26.70-30.60°C, 8.5-10.20mg/l and 8.0-8.60, respectively (Table 7).

Table 7. Mean water quality parameters observed during the experimental period fed with potato enriched feed

Parameters	Value range
Temperature	26.70-30.60°C
pH	8.0-8.60
Dissolved Oxygen (DO) (mg/l)	8.5-10.20mg/l

11.1.2 Evaluation of the efficiency and suitability of whole wheat flour on the growth and survival rate of Rohu (*Labeo rohita*)

One hundred eighty fingerlings of initial weight 1.74 ± 0.00 g were released in twelve aquariums at the same stocking density (268 fingerlings/ m^3). In this experiment different levels of whole wheat flour viz. Diet 1: 0% (control), Diet 2: 5% whole wheat flour, Diet 3: 10% whole wheat flour and Diet 4: 15% whole wheat flour were used for the treatment 1, 2, 3, and 4, respectively with three replications in each. All four diets had a constant inclusion level of the following ingredients: fish meal 30%, rice bran 30%, mustard oil cake 12%, molasses 5%, soybean oil 4% and vitamin and mineral premix 1%. Feeds were supplied 5% body weight twice daily in the morning at 9.00 am and in the afternoon at 5.00 pm throughout the experimental period. Sampling was done by using digital portable balance and fortified push net at 7 days interval throughout the study period to assess different growth parameter. Water quality parameters were within the acceptable range during the study period. Final weight (g), weight gain (g), percent weight gain (%), specific growth rate (%/day) and protein efficiency ratio (PER) varied from 3.03 ± 0.42 to 3.95 ± 0.11 g, 1.29 ± 0.44 to 2.21 ± 1.26 g, 74.28 ± 0.593 to $127.06 \pm 4.04\%$, 0.88 ± 0.05 to $1.30 \pm 0.03\%$ /day and 0.69 ± 0.06 to 0.94 ± 0.02 , respectively. Highest FCR (3.43 ± 0.29) was found in T_1 and the lowest FCR (2.42 ± 0.13) in T_4 . The highest PER (0.94 ± 0.02) was found in T_4 and the lowest PER (0.69 ± 0.06) in T_1 . The survival rate was found 100% in every treatment. This indicates that water quality parameters were within the acceptable range during the study period. Best growth performance was found in T_4 followed by T_3 , T_2 and T_1 .

11.1.3.1 Growth performances of *L. rohita*

The growth performances of *L. rohita* in terms of initial weight (g), final weight (g), weight gain (g), percent weight gain (%), specific growth rate (%/day), feed conversion ratio, feed conversion efficiency and protein efficiency ratio were calculated at the end of the experiment.

Table 8. Effect of different treatments on growth performance, feed utilization and survival rate of Rohu (*L. rohita*) fed with flour enriched diet in aquarium trial during the study period.

Variable parameters	T ₁ (0% wheat flour)	T ₂ (5% wheat flour)	T ₃ (10% wheat flour)	T ₄ (15% wheat flour)	LSD	Level of sign.
Initial weight (g)	1.74(±0.00)	1.74(±0.00)	1.74(±0.00)	1.74(±0.00)	0.00	NS
Final weight (g)	3.03(±0.10) c	3.89(±0.09)a	3.73(±0.19)b	3.95(±0.07)a	0.13	**
Weight gain (g)	1.29(±0.10) c	2.45(±0.08)a	1.99(±0.19)b	2.21(±0.07)a	0.13	**
% weight gain	74.28(±5.93) c	123.75(±4.96)a	114.58(±10.79) b	127.06(±4.04)a	7.54	**
SGR (%/day)	0.88(±0.05) b	1.28(±0.04)a	1.21(±0.08)a	1.30(±0.03)a	0.06	**
FCR	3.43(±0.29) a	2.43(±0.04)b	2.58(±0.23)b	2.42(±0.13)b	0.22	**
FCE	0.29(±0.03) b	0.41(±0.01)a	0.39(±0.04)a	0.41(±0.02)a	0.03	**
PER	0.69(±0.06) b	0.95(±0.03)a	0.87(±0.08)a	0.94(±0.02)a	0.06	*
Survival rate (%)	100.00(±0.00)	100.00(±0.00)	100.00(±0.00)	100.00(±0.00)	0.00	NS

Values given in bracket are standard deviation. The values in the same row having similar letter (s) do not differ significantly otherwise differ significantly ($p < 0.05$) as per Duncan Multiple Range Test (Duncan, 1955). NS=Not significant, * significant in 5%, ** significant in 1% significance level.

11.1.3.1.1 Initial weight (g)

The initial average weight of individual *L. rohita* in different treatments was 1.74g (Table 8).

11.1.3.1.2 Final weight (g)

The mean final weight of individual *L. rohita* in different treatments varied from 3.03 g to 3.95g (Figure 16). The mean weight gain (g) in T₄ was found highest followed by T₂, T₃, and T₁, respectively (Table 8).

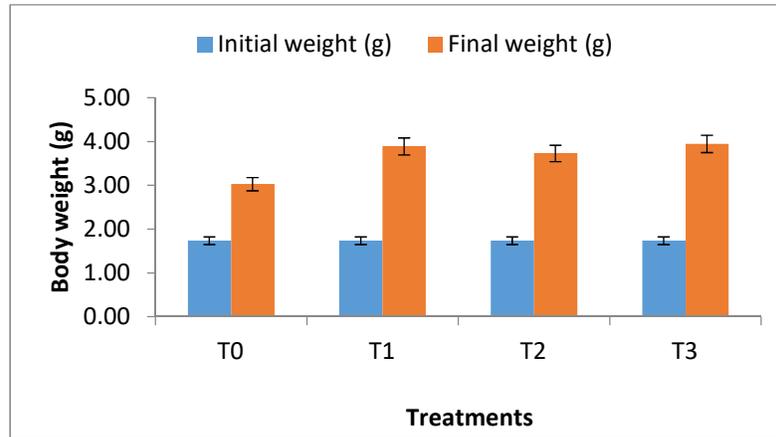


Figure 16: Mean final weight (g) of *L. rohita* fed wheat flour enriched diet in different treatments during the experimental period.

11.1.3.1.3 Weight gain (g)

The mean weight gain of individual *L. rohita* in different treatments ranged from 1.29g to 2.21g (Figure 17). The mean weight gain of experimental fish was found highest in T₄ followed by T₂, T₃ and T₁, respectively (Table 8).

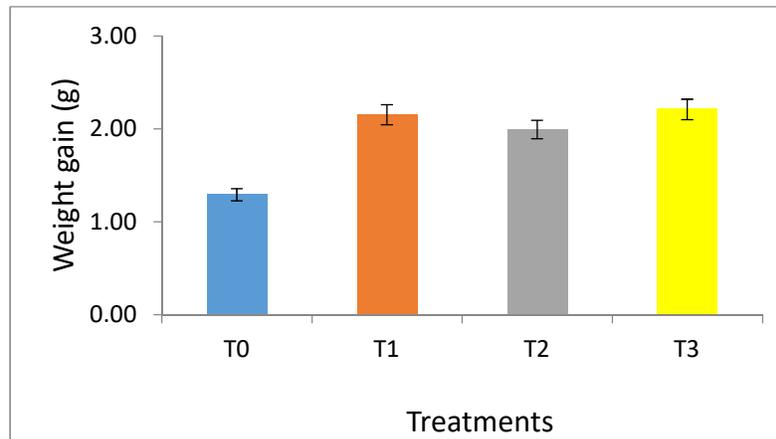


Figure 17. Mean weight gain (g) of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.4 Percent weight gain (%)

The percent weight gain (%) of 15 fish in different treatments ranged from 74.28% to 127.06% (Figure 18). The highest percent weight gain (%) was found in treatment T₄ followed by T₂, T₃ and T₁, respectively. There was significant ($p < 0.05$) variation of percent weight gain between T₁ and T₄; T₂ and T₃ but no significant variation observed between T₄ and T₂ (Table 8).

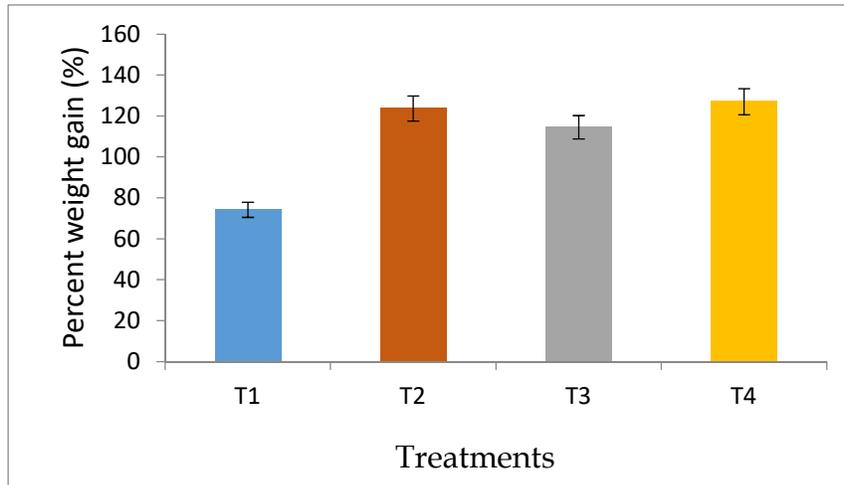


Figure 18. Mean percent weight gain (g) of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.5 Specific growth rate (%/day)

The specific growth rate (%/day) ranged from 0.88% to 1.30% /day (Figure 19). The highest specific growth rate (1.30%/day) was found in T₄ followed by T₂, T₃, T₁, respectively. There was significant ($p < 0.05$) variation of specific growth rate between T₁ and T₄; T₂ and T₃ but no significant variation ($p \geq 0.05$) found between T₁ and T₃; T₂ and T₄ (Table 8).

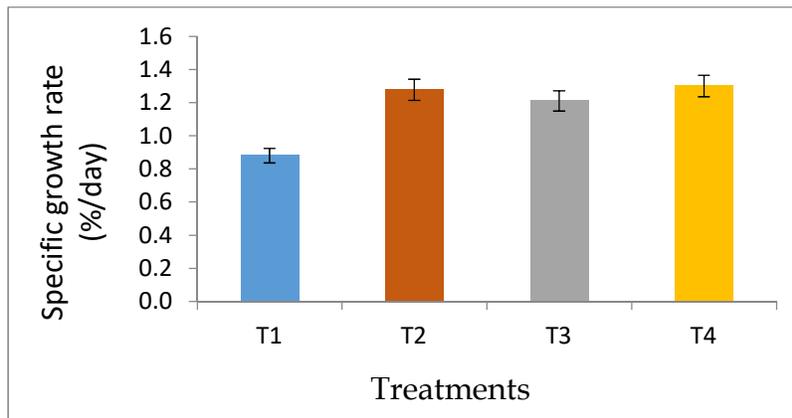


Figure 19. Mean Specific growth rate (%/day) of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.6 Feed conversion ratio (FCR)

Mean food conversion ratio in different treatments ranged from 2.42 to 3.43 (Figure 20). The highest FCR was obtained in T₁ followed by T₄, T₃, and T₂, respectively. There was no significant ($p \geq 0.05$) variation in mean food conversion ratio among T₂, T₃ and T₄ but has significant variation ($p < 0.05$) between T₁ and other treatments (Table 8).

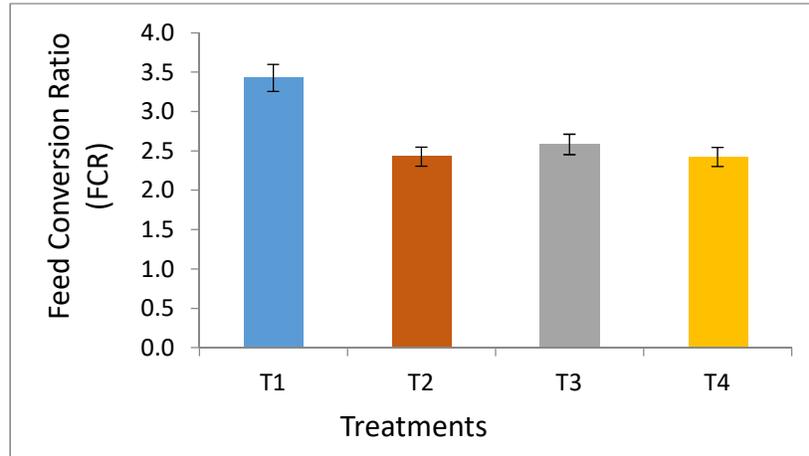


Figure 20. Mean Feed conversion ratio of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.7 Feed conversion efficiency (FCE)

Mean food conversion efficiency in different treatments ranged from 0.29 to 0.41 (Figure 21). The highest FCE was obtained in treatment T₄ followed by T₂, T₃ and T₁, respectively. There was no significant ($p \geq 0.05$) variation in mean food conversion ratio among T₂, T₃ and T₄ but has significant variation ($p < 0.05$) between T₁ and others (Table 8).

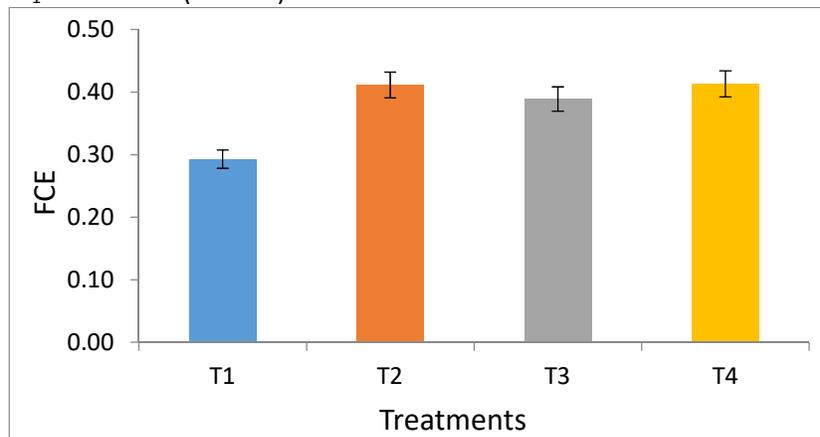


Figure 21. Mean Feed conversion efficiency of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.8 Protein efficiency ratio (PER)

Mean protein efficiency ratio (PER) in different treatments varied from 0.69 to 0.95 (Figure 22). The highest protein efficiency ratio (PER) was found in T₂ followed by T₄, T₃, and T₁, respectively. There was significance ($p < 0.05$) difference between T₁ and T₄; T₂ and T₃ but no significance variation between T₂ and T₄; T₃ and T₄; T₃ and T₁ (Table 8).

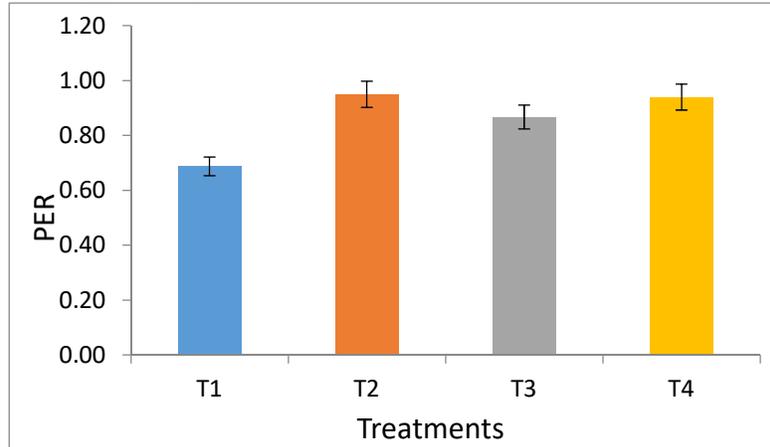


Figure 22. Mean Protein efficiency ratio of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.1.9 Survival rate (%)

The mean survival rate (%) of *L. rohita* under different treatments was 100%. (Table 4). There was no significant ($p \geq 0.05$) variation in survival rate of *L. rohita* in four treatments (Figure 23).

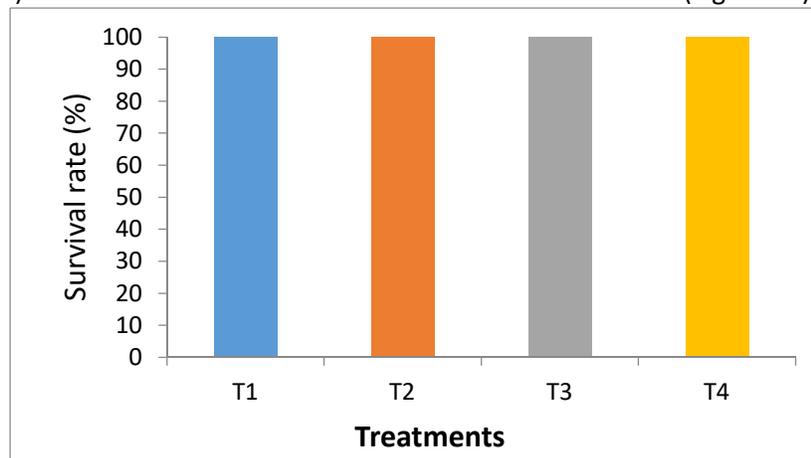


Figure 23. Mean Survival rate of *L. rohita* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.3.2 Water quality parameter

The water quality parameters such as temperature, dissolved oxygen and pH of different tank were measured throughout the experimental period. The range of temperature, dissolved oxygen and pH were from 27.6-29.63°C, 7.98-8.20mg/l and 8.3-9.4 (Table 9).

Table 9. Mean water quality parameters observed during the experimental period fed with wheat flour enriched feed for *L. rohita*

Parameters	Value range
Temperature (°C)	27.6 – 29.63
pH	7.98 – 8.20
Dissolved Oxygen (DO) (mg/l)	8.3 – 9.4

11.1.3 Efficacy of whole wheat flour as prebiotic compound on the growth and survival rate of Catla (*Catla catla*)

180 fingerlings (individual initial weight 6.85±0.00g) were released at the same stocking density (12 fingerlings per aquarium). In this experiment Diet 1: 0% whole wheat flour, Diet 2: 5% whole wheat flour, Diet 3: 10% whole wheat flour and Diet 4: 15% whole wheat flour were used for the treatment 1, 2, 3, and 4, respectively with three replications. All four diets having a constant inclusion level of the following ingredients: fish meal 30%, rice bran 30%, mustard oil cake 12%, molasses 5%, soybean oil 4% and vitamin and mineral premix 1%. Feeds were supplied @ 5% body weight twice daily in the morning at 9.00 am and in the afternoon at 5.00 pm throughout the experimental period. Sampling was done at 7 days interval throughout the study period to estimate the growth performance. Water quality parameters were maintained within the suitable range. Final weight (g), weight gain (g), percent weight gain (%), specific growth rate (%/day) and protein efficiency ratio (PER) varied from 13.49±0.27 to 15.63±0.88, 6.64±0.27 to 8.78±0.88, 96.95±3.88 to 128.11±12.79, 1.08±0.03 to 1.31±0.09, respectively. The gut microbiota of catla fish in case of TSA agar media ranged from 1.1~2.5×10⁵ to 3.3~7.2×10⁶ CFU/mL and in case of MRS agar media 1.0~3.0×10³ to 3.4~5.2×10⁴ CFU/mL were estimated. The highest range of bacterial colony was found in treatment 4 and the lowest bacterial colony was found in treatment 1. The growth performance was highest in treatment 3 followed by treatment 4, treatment 1, treatment 2 and the lowest FCR (2.28±0.25) was revealed in treatment 3 whereas the highest FCR was found in treatment 2 (2.99±0.14). The survival rate was 100% in all treatments.

Growth parameters of *C. catla* after feeding different diet such as Diet 1: 0% whole wheat flour, Diet 2: 5% whole wheat flour, Diet 3: 10% whole wheat flour, Diet 4: 15% whole wheat flour are as follows:

Table 10. The effects of different treatments on growth performance, feed utilization and survival rate of catla fish (*C. catla*) reared wheat flour enriched feed in aquaria during the study

Variable parameters	T ₁ (0% wheat flour)	T ₂ (5% wheat flour)	T ₃ (10% wheat flour)	T ₄ (15% wheat flour)	LSD	Level of sign.
Initial weight (g)	6.85 ± 0.00	6.85 ± 0.00	6.85 ± 0.00	6.85 ± 0.00	0.00	ND
Final weight (g)	13.82 (±0.34) b	13.49(±0.27)b	15.63(±0.88)a	14.46(±0.13)b	0.54	**
Weight gain (g)	6.97 (± 0.34)b	6.64(±0.27)b	8.78(±0.88)a	7.61(±0.13)b	0.54	**
%weight gain	101.78(±5.02) c	96.95(±3.88)c	128.11(±12.79)a	111.03(±1.86)b	7.82	**
SGR (%/day)	1.11(±0.04)bc	1.08(±0.03)c	1.31(±0.09)a	1.19(±0.01)b	0.06	**
FCR	2.99(±0.14)a	2.95(±0.12)a	2.38(±0.25)b	2.72(±0.05)a	0.17	**
FCE	0.34(±0.02)b	0.34(±0.01)b	0.42(±0.04)a	0.37(±0.01)b	0.03	**
PER	0.79 (±0.04)b	0.79 (±0.03)	0.94 (±0.09)a	0.85(±0.01)ab	0.06	**
Survival rate (%)	100(±0.00)	100(±0.00)	100(±0.00)	100(±0.00)	0.0	ND

Values given in bracket are standard deviation. The values in the same row having similar letter (s) do not differ significantly otherwise differ significantly ($p < 0.05$) as per Duncan Multiple Range Test (Duncan, 1955). NS=Not significant, * significant in 5%, ** significant in 1% significance level.

11.1.4.1 Growth performances of *C. catla*

The growth performances of *C. catla* in terms of initial weight (g), final weight (g), weight gain (g), percent weight gain (%) and specific growth rate (%/day) were calculated at the end of the experiment.

11.1.4.1.1 Initial weight (g)

The initial mean weight of *C. catla* in different treatments was 6.85±0.00g (Table 10).

11.1.4.1.2 Final weight (g)

The mean final weight of *C. catla* in different treatments varied from 13.49g to 15.63g. The mean weight gain (g) in T₃ was found highest followed by T₄, T₁, and T₂, respectively (Figure 24 and Table 10).

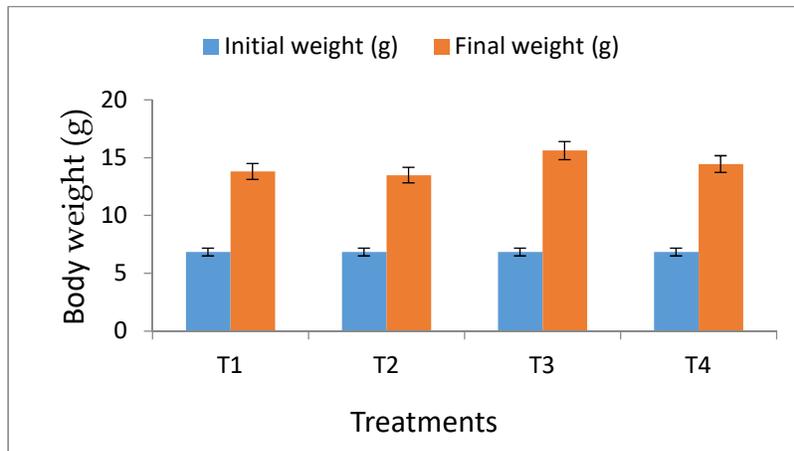


Figure 24: Mean weight of *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.3 Weight gain (g)

The mean weight gain of *C. catla* in different treatments ranged from 6.64g to 8.78g. The mean weight gain of experimental fish was found highest in T₃ followed by T₄, T₁ and T₂, respectively. There was significant ($p < 0.05$) variation of weight gain between T₃ with other treatments but no significant variation found among T₁, T₂, T₄ (Figure 25 and Table 10).

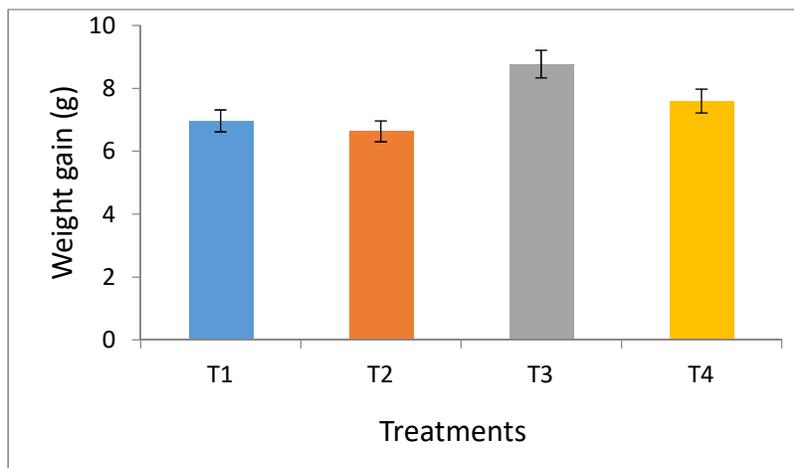


Figure 25: Mean weight gain (g) of *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.4 Percent weight gain (%)

The mean percent weight gain (%) of fish in different treatments ranged from 96.95% to 128.11% (Figure 26). The highest percent weight gain (%) was found in T₃ followed by T₄, T₁ and T₂, respectively. There was significant ($p < 0.05$) variation of percent weight gain between T₃ with other treatments but without significant variation among T₁, T₂, T₄ (Table 10).

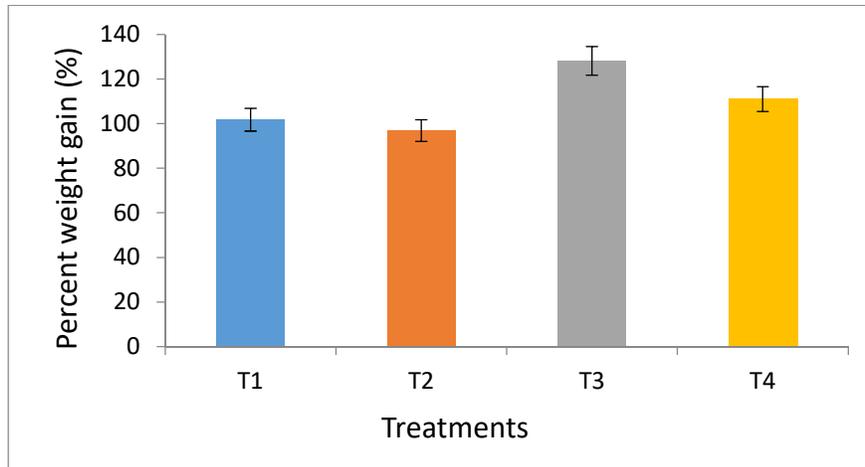


Figure 26: Mean percent weight gain (g) of *C. catla* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.5 Specific growth rate (%/day)

The specific growth rate (%/day) ranged from 1.08% to 1.31% /day (Figure 27). The highest specific growth rate (1.31% /day) was found in T₃ followed by T₄, T₁, T₂, respectively. There was significant ($p < 0.05$) variation of specific growth rate among the four treatments (Table 10).

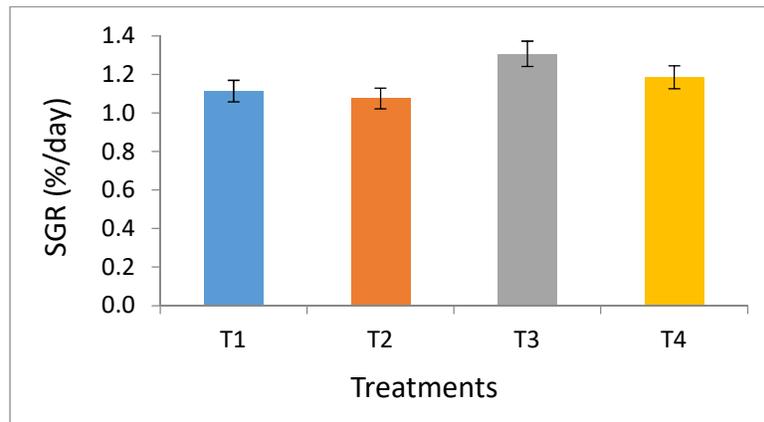


Figure 27: Mean SGR (%/day) of *C. catla* fed by wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.6 Feed conversion ratio

Mean food conversion ratio (FCR) in different treatments was ranged from 2.38 to 2.99 (Figure 28). The highest FCR was obtained in T₁ followed by T₂, T₄, and T₃, respectively. The significantly ($p < 0.05$) lowest FCR was found in T₃ when compared with other treatments and there was no significant ($p > 0.05$) variation in mean FCR among T₁, T₂ and T₄ (Table 10).

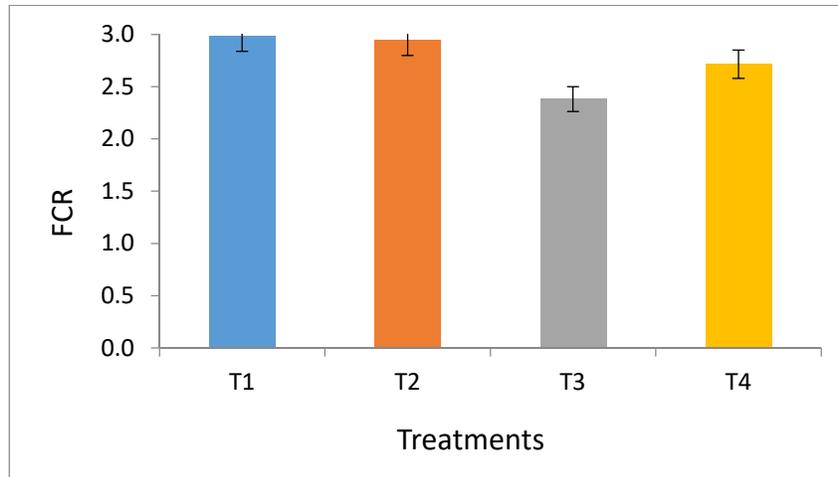


Figure 28: Mean FCR of *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.7 Feed conversion efficiency

Mean food conversion efficiency (FCE) in different treatments was ranged from 0.34 to 0.42 (Figure 29). The highest FCE was obtained in T_3 . There was no significant ($P>0.05$) variation in mean FCE among T_1 , T_2 and T_4 but had significant variation ($p<0.05$) between T_3 with others (Table 10).

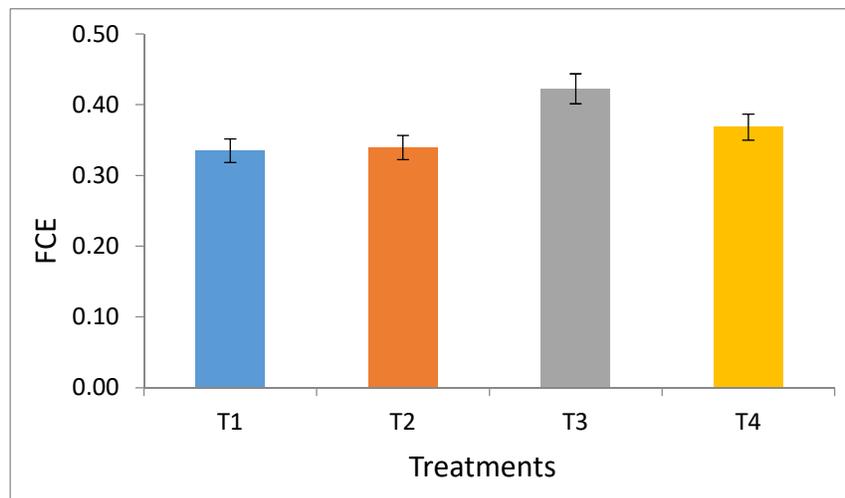


Figure 29: Mean FCE of *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.8 Protein efficiency ratio

Mean protein efficiency ratio (PER) in different treatments varied from 0.79 to 0.94 (Figure 30). The significantly ($p<0.05$) highest PER was found in treatment T_3 . Although PER value did not significantly ($p>0.05$) varied among the T_1 , T_2 and T_4 treatments (Table 10).

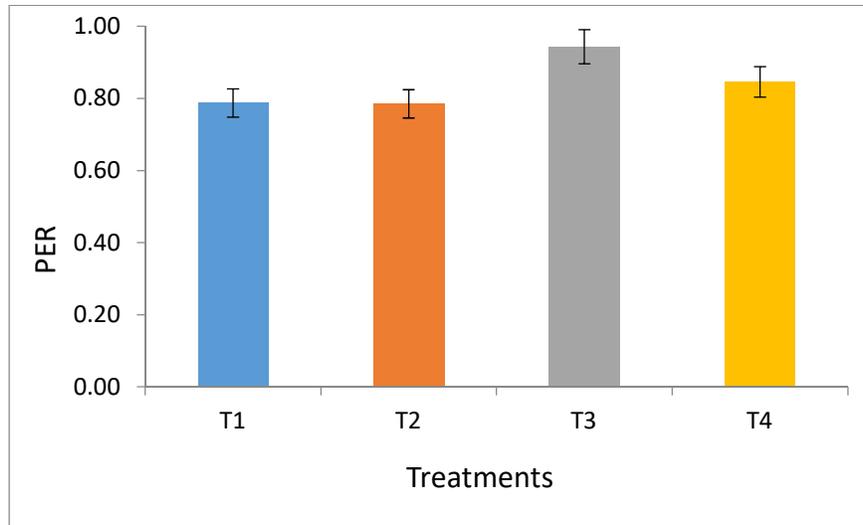


Figure 30: Mean PER *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.1.9 Survival rate (%)

The mean survival rate (%) of *C. catla* under different treatments was 100% (Figure 31). There was no significant ($p > 0.05$) variation obtained in survival rate of *C. catla* among four treatments (Table 10).

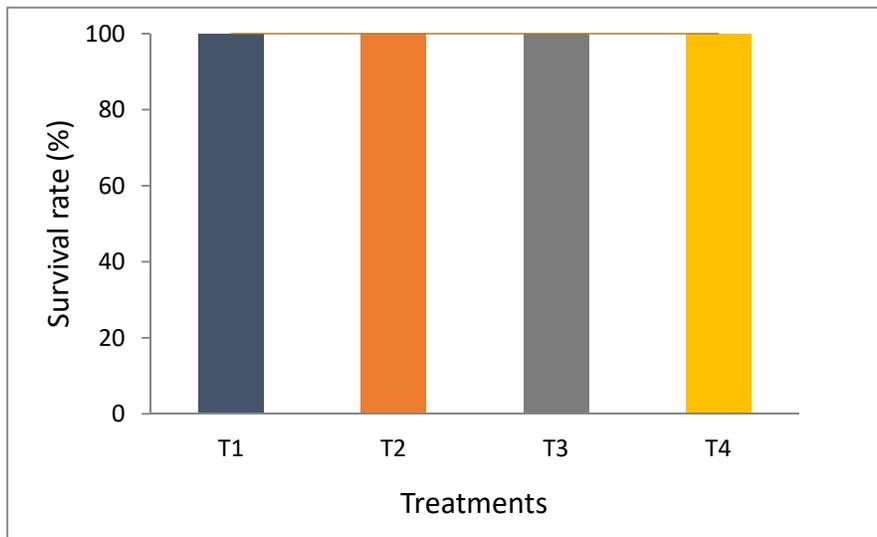


Figure 31: Mean survival rate of *C. catla* fed wheat flour enriched feed in different treatments during the experimental period.

11.1.4.2 Gut microbiota of *C. catla* in TSA and MRS agar media in different treatments

In this experiment the gut microbiota of *C. catla* was measured for different treatments after rearing 63 days in aquaria (Plate 5, 6). In case of TSA agar media the highest range ($3.3\sim 7.2\times 10^6$ CFU/mL) was found in T_4 which contained 15% whole wheat flour followed by T_3 , T_2 , T_1 , respectively and in case of MRS agar media the highest range was also found in T_4 ($3.4\sim 5.2\times 10^4$ CFU/mL) followed by T_3 , T_2 , T_1 , respectively. In both agar media the lowest bacterial colony was found in T_1 (control) which contained 0% whole wheat flour in diet composition (Table 11).



Plate 5: Collection of gut microbiota from *C. catla*



Plate 6. Analysis of gut microbiota from *C. catla*

Table 11. Plate count of *C. catla* gut microbiota in TSA and MRS agar media (CFU/mL) fed with wheat as prebiotic compound after 63 days of rearing in aquaria

Treatment	TSA agar media (CFU/mL)	MRS agar media (CFU/mL)
Treatment 1	$1.1 \sim 2.5 \times 10^5$	$1.0 \sim 3.0 \times 10^3$
Treatment 2	$4.7 \sim 6.8 \times 10^5$	$4.2 \sim 10.0 \times 10^3$
Treatment 3	$1.8 \sim 9.1 \times 10^5$	$0.82 \sim 32.0 \times 10^3$
Treatment 4	$3.3 \sim 7.2 \times 10^6$	$3.4 \sim 5.2 \times 10^4$

The effects of prebiotic on survival rate and fish growth performance of *L. rohita* were observed in the present study in laboratory condition. Fish fed with different % of prebiotic (potato powder) enriched diet showed different survival rate and growth performance during the experiment.

In this experiment, it was observed that highest final weight gain (2.73 ± 0.40 g) was recorded in T_4 . Another treatment T_1 , T_2 and T_3 showed the lower growth of *L. rohita* compared to T_4 . This result showed the positive impact of the prebiotic compound used in formulated feed.

The weight gain (g) of fishes were 1.29 ± 0.25 , 2.59 ± 0.31 , 1.95 ± 0.23 , and 2.73 ± 0.40 ; percent weight gain (g) were 74.32 ± 14.17 , 149.04 ± 17.55 , 112.45 ± 13.23 and 157.01 ± 22.77 ; SGR (% per day) were 0.38 ± 0.06 , 0.63 ± 0.05 , 0.52 ± 0.05 and 0.65 ± 0.06 in T_1 , T_2 , T_3 and T_4 , respectively were recorded in this experiment. The highest and lowest value of weight gain (g), percent weight gain and SGR (% per day) were found at T_4 and T_1 , respectively. Ali and Salim (2004) noted that *Labeo rohita* gained 2.63 ± 0.45 g body weights when fed sunflower meal, which was higher than the weight gained by hybrids (1.62 ± 0.05). Sahzadi *et al.* (2006) observed better growth in hybrid (*Catla catla* \times *Labeo rohita*) on sunflower meal (1.62 ± 0.0 g) than cotton seed meal (1.61 ± 0.01 g) and bone meal (1.52 ± 0.0 g).

Eidelsburger and Kirchgessner (1994) reported that calcium format alone or in combination with other acids when given at the rate of 0.5 and 1.5 %, increased FCR and growth performance up to 35 days of age. Benedetto (2003) also observed mix of organic acids (ACIDLAC) used as a replacer of growth

promoters (AGPs) and improved production performance along with other beneficial effects. Mairoka *et al.* (2004) also reported that mixture of organic acids can be effectively used as a substitution of antibiotic growth promoters (AGPs) for improved physiological performance. Savage *et al.* (1997) concluded from a dose responsive study that MOS @ 0.11 %, maximized weight gain up to 0-8 weeks of age. Stanley *et al.* (2004) found same type of effect with supplementation of 0.1 % MOS on body weight gain. Parks *et al.* (2001) reported from a study with MOS that MOS may be utilized as an alternative to AGPs to improve turkey performance.

The mean survival rate for *L. rohita* in the present study was $97.78\% \pm 2.22$ at T₁, but 100% survival rate at prebiotic treated group (T₂, T₃ and T₄).

Keramat (2015) reported that the addition of 1 g kg⁻¹ immunogen as a prebiotic improves growth performance and survival rate of *Rutilus kutum*. These are similar to results of Li and Gatlin (2005), Staykov *et al.* (2007) and Mohajer *et al.* (2010), who observed higher feed efficiencies in hybrid striped bass, rainbow trout, and *H. huso* fed Grobiotic® prebiotic, mannan-oligosaccharide, and immunogen, respectively.

The feed conversion ratio (FCR) was recorded in this experiment were 3.49 ± 0.47 , 2.12 ± 0.22 , 2.61 ± 0.20 and 2.15 ± 0.15 in T₁, T₂, T₃ and T₄, respectively. The highest and lowest FCR value was recorded in T₁ and T₂, respectively. The food conversion efficiency (FCE) in T₁, T₂, T₃ and T₄ were 0.29 ± 0.04 , 0.48 ± 0.05 , 0.38 ± 0.03 and 0.57 ± 0.04 , respectively. The highest and lowest FCE value was recorded in T₂ and T₁, respectively. Tarnchalanukit *et al.* (1983) estimated the FCR value of *C. batrachus* to be 1.24-1.32 in circular tank receiving high quality feed. Azimuddin (1998) investigated FCR from 1.73 to 2.04 in three months formulated feed feeding trial near the fisheries faculty building in Bangladesh Agricultural University, Mymensingh, which is higher than the value of present experiment.

Sahzadi *et al.* (2006) observed comparatively higher FCR on sunflower meal (1.78 ± 0.05) than cottonseed meal (2.17 ± 0.01) in hybrid (*Catla catla* × *Labeo rohita*). FCR for sunflower meal (7.61 ± 0.45) was higher than for rice polish (8.16 ± 0.12) fed fish.

The protein efficiency ratio (PER) was recorded in this experiment were 0.68 ± 0.09 , 1.09 ± 0.11 , 0.87 ± 0.07 and 1.06 ± 0.07 in T₁, T₂, T₃ and T₄, respectively. The highest and lowest PER value was recorded in T₂ and T₁, respectively. The FCR, FCE, PER results indicated that supplementing diets with the prebiotics significantly improved protein utilization in *L. rohita*. Lara-Flores *et al.* (2003) showed that the same results in which the addition of prebiotics improved feed utilization in practical terms. This means that prebiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction.

Present research interest was directed to formulate a low cost diet which has significant impact on growth performance of *C. catla* with finding out of required amount feed will enable us to reduce the feed wastage.

In this experiment effect of potato on the growth performance of *C. catla* in aquarium was investigated. Compared with other treatments, growth performance of *C. catla* was significantly ($P < 0.05$) higher (5.78g) in treatment-4 which was provided with higher levels of potato (15%) feed whereas lower in T₁ (control). Ali and Salim (2004) used balanced feed, whereas in the present study single ingredient was used in the experiment. Ali and Salim (2004) noted that *Labeo rohita* gained 2.63 ± 0.45 g body weights on sunflower meal, which was higher than the weight gained by hybrids (1.62 ± 0.05) in their study. Sahzadi *et al.* (2006) observed better growth in hybrid (*Catla catla* × *Labeo rohita*) on sunflower meal (1.62 ± 0.0 g) than cotton seed meal (1.61 ± 0.01 g) and bone meal (1.52 ± 0.0 g).

The specific growth rate (SGR%/day) in treatment 1 (control) was 0.55 ± 0.03 , treatment 2 was 0.78 ± 0.02 , treatment 3 was 0.86 ± 0.00 and treatment 4 (15% potato) was 0.91 ± 0.03 . In present study, specific growth rate (SGR) varied from (0.55 ± 0.03) % to (0.91 ± 0.03) %.

Popma (1982) who observed that Nile tilapia can digest over 70% of the energy of raw corn starch. Also, the relatively good SGR and body condition factor in *O. niloticus* used for this study fed grains sources at 57% inclusion level is suggestive that *O. niloticus* might also be able to regulate amyloytic activity like carp. Hasan *et al.* (1982) found more or less similar result from the present study. The highest SGR value was found at T₃ and lowest was observed at T₁.

In the present study, the feed conversion ratio (FCR) in treatment 1 (control) was 6.18 ± 0.10 , treatment 2 (5% potato) was 4.07 ± 0.16 , treatment 3 (10% potato) was 3.84 ± 0.18 and treatment 4 (15% potato) was 3.59 ± 0.18 . The highest FCR value was recorded in T₁ (Control). The lowest FCR value was recorded in T₄ (15% potato). Food is one of the important factors promoting growth and the feed conversion ratio (FCR) is an appropriate way to judge the acceptability and suitability of artificial feed for fish.

Inayat and Salim (2005), found higher FCR (1.70) in *Cirrhinus mrigala* when fed on soybean meal and lowest (3.36) when fed on maize. Saeed *et al.* (2005) reported that FCR values in *Labeo rohita* decrease in following order blood meal (2.31 ± 0.87), followed by soybean meal (3.46 ± 0.69) and corn gluten meal (60%) (5.00 ± 1.27). Sahzadi *et al.* (2006) observed comparatively higher FCR on sunflower meal (1.78 ± 0.05) than cottonseed meal (2.17 ± 0.01) in hybrid (*Catla catla* × *Labeo rohita*). FCR for sunflower meal (7.61 ± 0.45) was higher than for rice polish (8.16 ± 0.12) fed fish. Same was reported by Ali and Salim (2004) who observed better FCR for sunflower meal, followed by fishmeal and rice polish.

In the present study the 100% survival rate was in all treatment. This result of 100% survival in four treatments indicated that potato supplementation had significant effect on survival rate of *C. catla*.

Ahmed *et al.* (2012) observed that 100% survival of *Labeo rohita* for different feeds. Haque and Ahmed (1993) reported survival of carp spawn in different pond were 70.07%, 71.44% and 58.32%, respectively. Wahab *et al.* (1995) found that the survival rate of all fish including Thai sarpunti was higher than (80%) in polyculture with native major carps. Kohinoor *et al.* (1993) reported the survival rate of Thai sarpunti ranged from 86.0% to 94% in monoculture system. The findings of the previous studies indicate that

In this experiment the gut microbiota of *C. catla* was measured for different treatments after rearing of 63 days in aquarium. The highest bacterial load in TSA agar media was 9.6×10^7 CFU/ml found in treatment 4 (15% potato) and lowest was 1.7×10^6 CFU/ml in treatment 1 (control) in case of the MSA agar media the highest bacterial load was 7.5×10^4 CFU/ml in treatment 2 (5% potato) and lowest was 5.7×10^2 CFU/ml in treatment 1 (control). The lowest bacterial colony in both agar media was found in treatment 1 (control).

Hovda *et al.* (2007) reported that culturable bacterial levels recovered on TSA agar plates from groups exposed to saline were relatively low, ranging from log 1.72 to 2.34 CFU g⁻¹. These values are low compared to autochthonous levels previously reported in Atlantic salmon and rainbow trout *Oncorhynchus mykiss* by Merrifield *et al.* (2009). Salinas *et al.* (2008) exposed to *C. divergens* in the ex vivo studies the same *C. divergens* strain was identified to dominate both the PI and DI after exposure. *C. divergens* levels were in the range of 10⁴-10⁶ CFUg⁻¹ intestine which indicates that the bacteria are able to populate and potentially colonize the intestinal mucus and out-compete other adherent bacteria after only one hour of exposure. These results are in accordance with corresponding studies in that lactic acid bacteria are able to colonize the intestine of Atlantic salmon after one hour exposure.

The water quality parameters play an important role for maintaining healthy environment for aquatic organisms. The water temperature monitored during the study period in the experimental tanks varied

from 26.3 to 29.7°C. Boyd (1982) reported that the range of water temperature from 26.06 to 31.97°C is suitable for fish culture. Hossain (2009) and Alam (2009) measured the water temperature in ponds of Agro-3 Farm; Trishal, Mymensingh ranged from 26.9 to 32.5°C. Hossain (2004) measured the water temperature in ponds of BAU campus, Mymensingh ranged from 29.4 to 33.0°C and 26.0 to 32.8°C, respectively. From the above statement, water temperature in aquarium was similar of pond temperature. The dissolved oxygen content from present experiment ranged from 8.3 to 9.4mg/l. Hossain (2009) and Alam (2009) measured the dissolved oxygen in ponds of Agro-3 arm, Trishal, Mymensingh ranged from 5.5 to 6.5 mg/l. Hossain (2009) and Alam (2009) measured the pH value in ponds of Agro-3 Farm, Trishal, Mymensingh ranged from 7.54 to 8.3 and 7.72 to 8.03, respectively. In this experiment pH value was recorded 7.98 to 8.20. Jena *et al.* (1998 a,b) assed that Physico-chemical parameters such as temperature (28 ± 1 °C), dissolved oxygen (6.7 ± 0.2 mg/L), pH (7 ± 0.5), total alkalinity (419 ± 18), total hardness (148 ± 10) and free carbon dioxide (7.8 ± 0.6 mg/L) in different treatments did not show any marked variations. The recorded parameters in the experiments were within the optimum range for fingerlings rearing.

Aquaculture is one of the fastest emerging food producing sectors of the world. World aquaculture has immensely grown during the last few years as well as becoming an economically significant zone. Various sectors of the aquaculture industry would benefit if cultured organisms were conferred with enhanced growth performance, feed efficiency and disease resistance. As such, the cost of medication and production costs could be reduced and consumer perceptions would be improved.

It has been documented in a number of terrestrial animals that microbiota of the gastrointestinal tract plays an important roles in affecting the nutrition and health of the host. To increase the fish production, improved techniques should be applied and management practices should be developed. On the other hand, artificial feed application is the most important technique to increase the production. Application of supplementary feed can play a vital role to increase the fish production. Dietary supplement such as probiotic, prebiotic and synbiotic provide nonspecific disease protection and also act as growth promoting factors. Dietary supplementation of prebiotics in fish and shellfish has investigated the following parameters: effect on growth, feed conversion, gut microbiota, cell damage/morphology, resistance against pathogenic bacteria and innate immune parameters. If the use of prebiotics leads to health responses becoming more clearly manifested in fish and shellfish, then prebiotics might have the potential to increase the efficiency and sustainability of aquaculture production.

From this point of view the experiment was carried out to estimation of the suitability and efficacy of potato and wheat flour as the prebiotic compounds on the growth performance and survival rate of *Labeo rohita* and *Catla catla*.

The result of the present study proves significant role of potato and wheat flour supplementation on the growth performance of *L. rohita* and the optimum inclusion level was 15%. Replacing 15% rice bran with 15% potato and whole wheat flour was quite satisfactory because of its nutritional content and path of its digestion. So it can be concluded that 15% potato and whole wheat flour has paramount importance in enhancing the production of *L. rohita* and recommended to incorporate with the feed.

The result of the present investigation also showed significant role of potato and wheat supplementation on the growth performance of *C. catla* and the optimum inclusion level were 15% and 10%, respectively. So it can be concluded that 15% potato and 10% whole wheat flour as prebiotic compound have maximum importance in enhancing the production of *C. catla* and recommended to incorporate with the feed.

Dietary supplementation of different feed additives e.g. probiotics usually in small quantities have been found to improve feed efficiency and growth performance of cultured fishes. So, probiotic used as alternatives to growth promoters but their combination strategy can be used to achieve good health and growth performance.

For better understanding more research is needed because potato and whole wheat flour supplement are good source of carbohydrate which ultimately effect on growth performance of *L. rohita* and *C. catla* and made economically viable and environmentally friendly which could be chosen by the feed manufacturers and fish farmer.

A few recommendations are:

- Further research should be carried out in pond system for better growth understanding.
- Inclusion level of potato and whole wheat flour for other important fish species should be determined.

12. Research highlight/findings (Bullet point – max 10 nos.):

- Development of Rohu and catla feed using potato and wheat as prebiotic compounds
- Increased bacterial load in gut content of Rohu and catla due to prebiotic properties of potato and wheat
- Higher growth performance viz. weight gain, percent weight gain, SGR and FCR were found due to prebiotic properties of potato and whole wheat flour.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	<u>Furniture</u> a) Executive Table b) Executive Chair c) Steel almira d) File cabinet <u>Camera, computer & accessories</u> a) Laptop b) UPS c) Camera	<u>74000.00</u> 20000.00 10000.00 24000.00 20000.00 <u>95000.00</u> 60000.00 10000.00 25000.00	<u>Furniture</u> a) Executive Table b) Executive Chair c) Steel almira d) File cabinet <u>Camera, computer & accessories</u> a) Laptop b) UPS c) Camera	20000.00 10000.00 24000.00 20000.00 60000.00 10000.00 25000.00	
(b) Lab & field equipment	<u>Lab. Equipments</u> a) Analytical balance b) Portable balance c) DO meter d) pH meter <u>Glasswares</u> <u>Chemicals</u>	<u>385000.00</u> 250000.00 90000.00 25000.00 20000.00 <u>250000.00</u>	<u>Lab. Equipments</u> a) Analytical balance b) Portable balance c) DO meter d) pH meter <u>Glasswares</u>	250000.00 90000.00 25000.00 20000.00 250000.00 370000.00	

		<u>370000.00</u>	Chemicals		
(c) Other capital items					

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
(a) Aquarium repair, renovation and maintenance	100000.00	Completed			
(b) Pond construction and management	300000.00	Completed			

3. Training/study tour/ seminar/workshop/conference organized: Not Applicable

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	371260	371260	371260	0	100	
B. Field research/lab expenses and supplies	1715843	1703234	1415842	287392	83	
C. Operating expenses	260034	218032	152960	65072	59	
D. Vehicle hire and fuel, oil & maintenance	200055	170046	200000	0	100	
E. Training/workshop/seminar etc.	00	00	00	0	00	
F. Publications and printing	137760	15,000	14250	750	10	
G. Miscellaneous	37866	37291	37291	0	100	
H. Capital expenses	1010849	1040847	964050	76797	95	
Grand Total	3733667	3773742	3155653	430011	68.38	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To determine the effects of Potato and Wheat as prebiotics on growth performance of <i>Labeo rohita</i> and <i>Catla catla</i>	Feeding potato and wheat enriched diet to Rohu and Catla to observe growth performance and survival rate.	Higher Growth Performance and survival rate of the experimental fish were observed and recorded.	Use of potato and wheat enriched diet enhanced growth performance and survivability of Rohu and Catla.
To evaluate Potato and Wheat enriched diet to increase the beneficial gut micro flora of Rohu and Catla	Culture and total counting of gut micro flora inoculated from experimental Rohu and Catla	Higher total counts of beneficial bacteria were observed in the fish fed with potato and wheat enriched diet.	Potato and wheat have the capacity to enhance the growth of beneficial bacteria in fish gut.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	1		
Journal publication	3		
Information development			
Other publications, if any: MS Thesis		4	i. EVALUATION OF THE SUITABILITY AND EFFICACY OF POTATO AS PREBIOTIC COMPOUND ON THE GROWTH PERFORMANCE OF ROHU (<i>Labeo rohita</i>) ii. ESTIMATION OF THE SUITABILITY AND EFFICACY OF POTATO AS PREBIOTIC COMPOUND ON THE GROWTH PERFORMANCE AND SURVIVAL RATE OF CATLA (<i>Catla catla</i>) iii. THE EFFICACY AND SUITABILITY OF WHOLE WHEAT FLOUR SUPPLEMENTATION ON THE GROWTH AND SURVIVAL RATE OF ROHU (<i>Labeo rohita</i>) iv. ESTIMATION OF THE EFFICACY OF WHOLE WHEAT FLOUR AS PREBIOTIC COMPOUND ON THE GROWTH PERFORMANCE AND SURVIVAL RATE OF CATLA (<i>Catla catla</i>)

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Development of fish feed using potato and wheat flour having prebiotic properties

ii. Generation of new knowledge that help in developing more technology in future

Wheat and potato had been applied as prebiotics for fish.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Prebiotic enriched fish feed to enhance growth performance of fish.

iv. Policy Support

Commercial production and dissemination of prebiotic enriched fish feed to promote growth and production of fishes in Bangladesh

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

- a) Workshop on mid-term review of research progress under CRG support, Fisheries Division, BARC, 10-11 April 2018.
- b) Annual review workshop on CRG sub-projects of Fisheries Division, BARC, 19-20 September 2018.

ii) Field Monitoring (time& No. of visit, Team visit and output):

- a) **Field monitoring** - by the Monitoring team of PIU-BARC, NATP-2 dated 7/3/2018 at BAURES and Research Laboratory of Aquaculture, BAU. The team expressed satisfaction on the research output.

H. Lesson Learned/Challenges (if any)

- i) Potato and whole wheat flour possess prebiotic properties.

I. Challenges (if any) N/A

Signature of the Principal Investigator
Date
Seal

Counter signature of the Head of the
organization/authorized representative
Date
Seal

J References:

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