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

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Abstract
This study aimed to evaluate the efficacy of whole wheat flour supplementation as prebiotic compound in feeds on the growth, survival rate and production of *C. catla*. A total of 144 fingerlings of mean initial weight of 6.85±0.45 g was stocked at the same stocking density (12 fingerlings per aquarium). Four different treatments (T1, T2, T3 and T4) each with three replications having different level of whole wheat flour such as 0% whole wheat flour (T1), 5% whole wheat flour (T2), 10% whole wheat flour (T3) and 15% whole wheat flour (T4). Feeds 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. Weekly weights recorded and feed supplied was used to compute the growth parameters. 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 $1.1\sim2.5\times10^5$ to $3.3\sim7.2\times10^6$ CFU/mL and in case of MRS agar media $1.0\sim3.0\times10^3$ to $3.4\sim5.2\times10^4$ 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. The result of present study indicate that prebiotic supplementation for catla has positive effects on the growth performance and the best result was obtained with a supplementation of 10% whole wheat flour.

Keywords: Efficacy, prebiotic, wheat flour, growth performance, survival rate

1. Introduction
Aquaculture is one of the world's promising and fastest developing food-producing sectors with the largest potential to accomplish growing demand of aquatic food (Pandiyan *et al*., 2013) [11]. Global aquaculture production (including aquatic plants) in 2016 was 110.2 million tonnes, with the first-sale value estimated at USD 243.5 billion and according to the latest statistical information from FAO, total global aquaculture production increased by 4.5 percent from 105.46 million ton (live weight equivalent) in 2015 to a new high of 110.21 million ton in 2016, with total production valued at US$ 243.26 billion and contribution of aquaculture to the global production of capture fisheries and aquaculture combined has risen continuously, reaching 46.8 percent in 2016, up from 25.7 percent in 2000 (FAO, 2018) [4]. Bangladesh has achieved 5th among the world's major aquaculture practicing countries in terms of fish and shrimp production (FAO, 2018) [4]. Intensification of aquaculture with high stocking densities, high food inputs and high organic load is said to be paralleled with a corresponding increase in the occurrence and spread of pathogenic and opportunistic bacteria causing infectious diseases. So there is a need for enhanced disease resistance, feed efficiency and growth performance of cultured fish species. Various types of feed additives e.g. immunostimulants, probiotics and prebiotics are usually used in small quantities to improve the immune status, feed efficiency and growth performance of finfishes and crustaceans (Ganguly *et al*., 2013) [5]. Prebiotics stimulates and activates the growth and metabolism of health promoting bacteria in the gastrointestinal tract (Manning and Gibson, 2004) [9]. The ultimate goal of using probiotic bacteria or prebiotic fibres in aquaculture is to improve the health and/or growth performance of fish by establishing beneficial bacterial community.

Thus, prebiotic fibers are believed to be a more practicable supplementation in fish cultures to stimulate the growth of beneficial bacteria (probiotic). Considering the above circumstances, the present study was designed to evaluate the efficacy of whole wheat flour supplementation as a prebiotic compound into the diets for *C. catla* and estimate the growth performance and survival rate of *C. catla.*

2. Materials and Methods

The experiment was carried out in the Wet Laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh between April and June 2018.

2.1 Experimental design

The experiment was carried out using 12 glass aquaria of 0.61 m × 0.31 m × 0.40 m in the Wet Laboratory of Bangladesh Agricultural University. Aeration was provided to the aquariums throughout the experimental period using aerators that replenish the amount of dissolved oxygen in the water and also to produce some current for the movement of food particles in the water Four treatments were used with three replicates. The fish in each of the aquarium was weighed weekly using an electronic balance. Fingerlings of *C. catla* of similar weight range and standard length was procured from Fish Hatchery Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The fingerlings were transported to the Wet Laboratory by using a plastic bag containing 70% water and 30% oxygen and were immediately placed in a 60 litres aquarium. The fingerlings were fed a control diet at every 9 am and 4 pm for seven days and were then starved for 24 hours prior to the start of the experiment before evenly stocked into the 12 aquariums at 12 fingerlings per aquarium.

2.2 Experimental diets

Locally procured feed ingredients were used to formulate the diet for the fingerlings throughout the experiment. The feed ingredients for all four diets had a constant inclusion level of: fish meal (FM) 30%, soybean meal (SBM) 20%, mustard oil cake (MOC) 12%, molasses 5.5%, vitamin premix 1%, mineral premix 1%, and chromic oxide 0.5%. Four graded levels of whole wheat flour 0%, 5%, 10% and 15% were included in the basal diet at the expense of rice bran. The ingredients used for preparation of basal experimental diets along with their proximate composition are shown in Table 1.

Table 1: Proximate composition analysis of different feed ingredients (dry basis)

Feed ingredients	Crude protein (%)	Crude Lipid (%)	Moisture (%)	Ash (%)	Crude fibre (%)	NFE
Fish meal	58.74	10.60	8.51	16.60	2.80	2.75
Rice bran	13.61	11.40	12.01	13.60	6.80	42.58
Whole wheat flour	16.13	5.60	13.14	13.40	6.95	44.78
Soybean meal	32.34	4.60	13.48	8.40	6.88	34.3

Each ingredient of the feed was ground to dust using a grinding machine. All the various components were mixed in their various ratios in order to produce the various diets needed for the experiment. The feed ingredients (finely ground and sieved) were weighed accordingly, moistened with water to form dough and pelletized using an electric meat mincer. The feeds were stored in airtight polythene bags at room temperature. The experimental diet samples were analyzed for proximate composition at the Fish Nutrition Laboratory of the Bangladesh Agricultural University, Mymensingh using the Association of Official Analytical Chemists official methods of analysis 15th edition, Washington DC (AOAC, 2000) [1]. Proximate composition of the three formulated feed is shown in Table 2.

Table 2: Proximate composition analysis of formulated feed (dry basis)

Diet	Moisture (%)	Crude Lipid (%)	Crude protein (%)	Ash (%)	Crude fibre (%)	NFE
Control	9.15	8.90	37.39	15.69	4.40	24.47
Diet 2	9.33	8.56	37.93	15.33	4.65	24.20
Diet 3	7.31	7.90	39.35	14.31	4.80	26.33
Diet 4	8.97	8.35	38.26	13.96	5.30	25.16

2.3 Fish feeding and aquarium management

The fish were fed at 5% of their body weight in two rations, during the morning at 9 am and the afternoon at 5 pm throughout the experiment. The rations were adjusted every week when new weights of the fish for various experimental aquariums were determined. Left over feed and faeces in each tank were siphoned every morning prior to feeding and replaced with fresh water.

2.4 Monitoring of water quality

Physico-chemical parameters such as temperature, dissolved oxygen and pH in the various aquariums were taken once per week during the early morning periods prior to siphoning and feeding throughout the experimental period.

2.5 Determination of growth and nutrient utilization

The weekly weights of fish and feed supplied was recorded and used to compute the growth nutrient utilization parameters. Weight of fish was taken weekly using digital balance (AND GULF, Dubai, model: GL-300). The growth and nutrient utilization parameters such as weight gain (g), percentage weight gain (%), specific growth rate (SGR, %/day), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (%) were calculated.

2.6 Isolation and enumeration of microbiota from experimental fish

The prebiotic treated catla (*C. catla)* were randomly sampled (2 fish/ treatment) for gut content analysis at the end of the experiment. After surface disinfection with 70% ethanol soaked cotton, the entire gut was carefully removed by sterile forceps. About 0.3 g gut from each fish was cut using sterile scissors and homogenized using sterile physiological saline (0.85%) at 4 ºC. The resultant aliquot was serially diluted, plated on tryptone soya agar (TSA) and de Man, Rogosa and Sharpe (MRS) agar media and L-shaped rod was used for

smooth spreading of diluted bacterial sample. Then the petri-dishes were incubated for 24 h at 37ºC to recover bacteria of gut samples. The bacterial populations of gut samples were expressed as number of colony forming units/mL (CFU/mL).

2.7 Data analysis
The collected data were statistically analyzed by one-way ANOVA with the help of SPSS to see whether the influence of different treatments on these parameters were significant or not. The means of different treatment were compared by DMRT (Duncan, 1955) [3] to test the significance of variation between the treatment means at $p<0.05$.

3. Results
3.1 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.

3.2 Initial weight (g)
The initial mean weight of *C. catla* in different treatments was 6.85±0.45g (Table 4).

3.3 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 T_3 was found highest followed by T_4, T_1, and T_2, respectively (Figure 1 and Table 3).

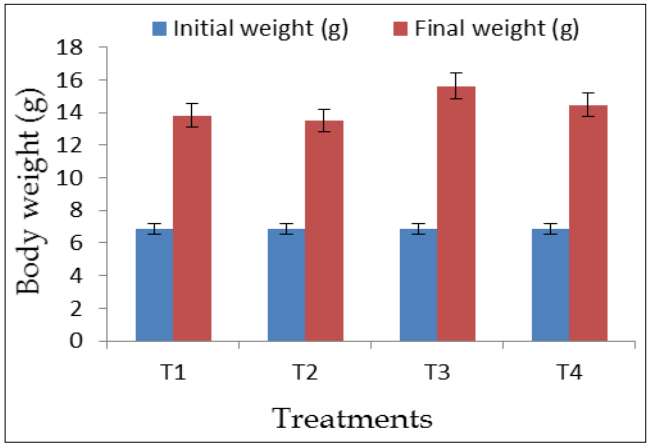

Fig 1: Mean weight variance of *C. catla*

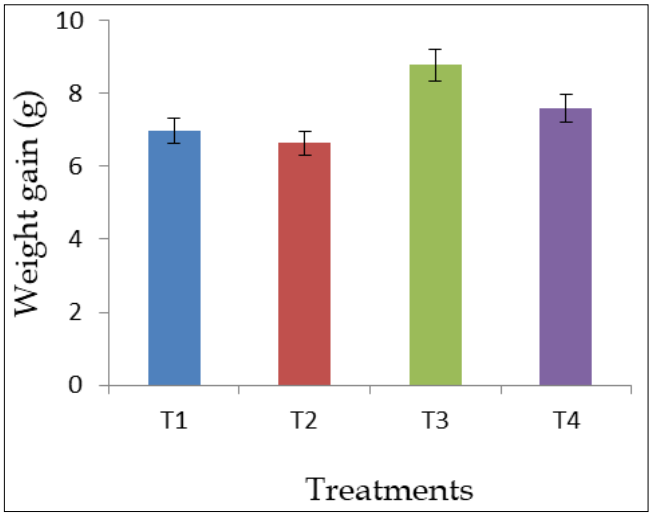

Fig 2: Mean weight gain (g) of *C.* catla

3.4 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 T_3 followed by T_4, T_1 and T_2, respectively. There was significance ($p<0.05$) variation of weight gain between T_3 with other treatments but no significance variation among T_1, T_2 , T_4 (Figure 2 and Table 3).

3.5 Percent weight gain (%)
The mean percent weight gain (%) of fish in different treatments ranged from 96.95% to 128.11%. The highest percent weight gain (%) was found in T_3 followed by T_4, T_1 and T_2, respectively. There was significance ($p<0.05$) variation of percent weight gain between T_3 with other treatments but no significance variation among T_1, T_2 , T_4 (Figure 3 and Table 3).

3.6 Specific growth rate (%/day)
The specific growth rate (%/day) ranged from 1.08% to 1.31% /day. The highest specific growth rate (1.31% /day) was found in T_3 followed by T_4, T_1, T_2, respectively. There was significant ($p< 0.05$) variation of specific growth rate among the four treatments (Figure 4 and Table 3).

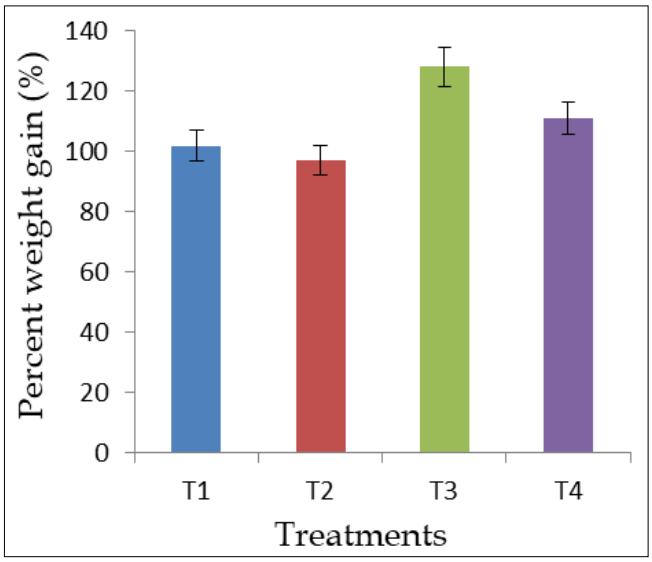

Fig 3: Mean percent weight gain (g) of *C. catla*

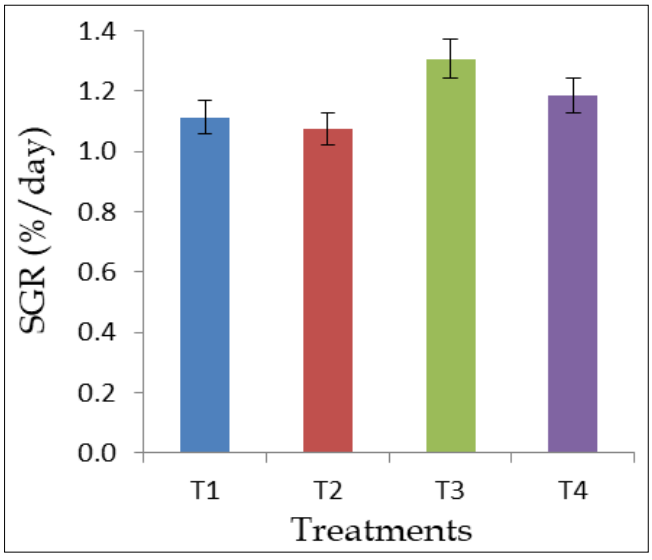

Fig 4: Mean SGR (%/day) of *C. catla*

3.7 Feed conversion ratio

Mean feed conversion ratio (FCR) in different treatments was ranged from 2.38 to 2.99. The highest FCR was obtained in T_1 followed by T_2, T_4, and T_3, respectively. The significantly ($p< 0.05$) lowest FCR was found in T_3 when compared with other treatments and there was no significant ($P>0.05$) variation in mean FCR among T_1, T_2 and T_4 (Figure 5 and Table 3).

3.8 Feed conversion efficiency

Mean feed conversion efficiency (FCE) in different treatments was ranged from 0.34 to 0.42. 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 has significant variation ($p< 0.05$) between T_3 with others (Figure 6 and Table 3).

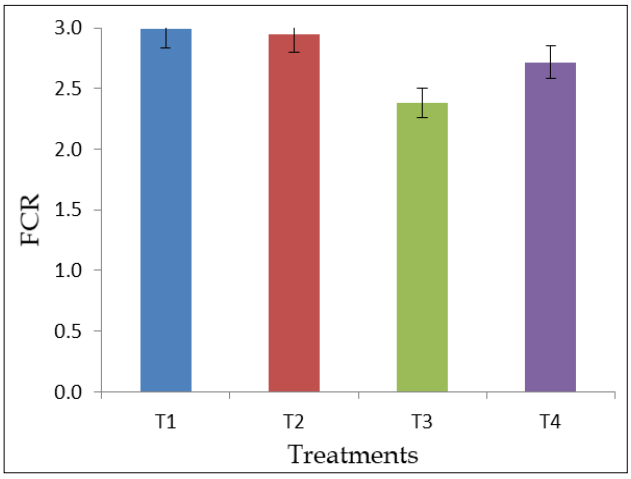

Fig 5: Mean FCR of *C. catla*

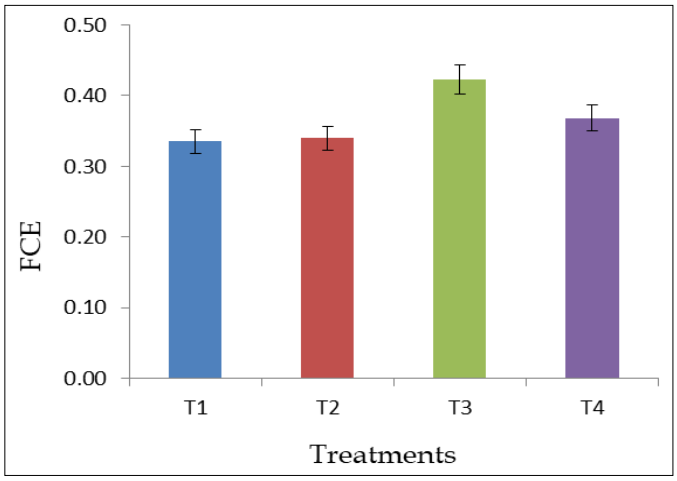

Fig 6: Mean FCE of *C. catla*

3.9 Protein efficiency ratio

Mean protein efficiency ratio (PER) in different treatments varied from 0.79 to 0.94. 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 (Figure 7 and Table 3).

3.10 Survival rate (%)

The mean survival rate (%) of *C. catla* under different treatments was 100%. There was no significant ($P>0.05$) variation in survival rate of *C. catla* among four treatments (Figure 8 and Table 4).

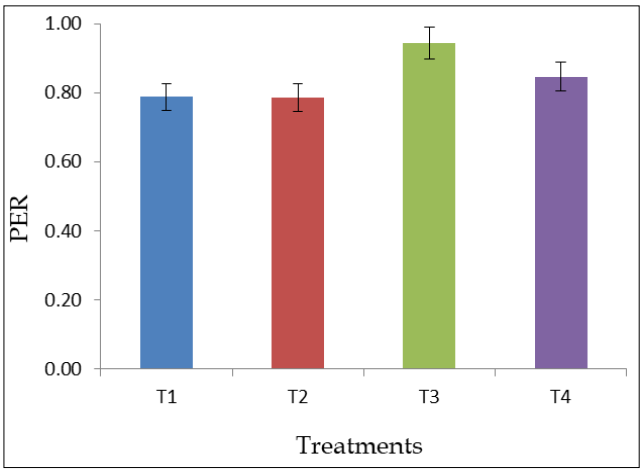

Fig 7: Mean PER *C. catla*

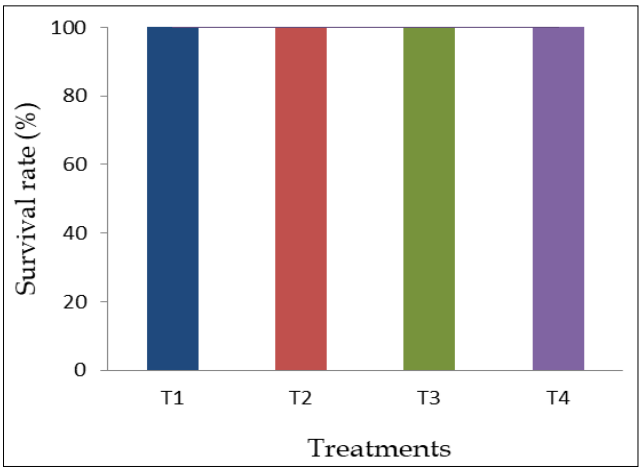

Fig 8: Mean survival rate of *C. catla*

Table 3: The effects of different treatments on growth performance, feed utilization and survival rate of catla fish (*C. catla*) reared in aquaria (Mean ± SE) during the study

Variable parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Level of sign.
Initial weight (g)	6.85 ± 0.45	6.85 ± 0.45	6.85 ± 0.45	6.85 ± 0.45	ND
Final weight (g)	13.82 (±0.34) b	13.49(±0.27)b	15.63(±0.88)a	14.46(±0.13)b	**
Weight gain (g)	6.97 (± 0.34)b	6.64(±0.27)b	8.78(±0.88)a	7.61(±0.13)b	**
%weight gain	101.78(±5.02)c	96.95(±3.88)c	128.11(±12.79)a	111.03(±1.86)b	**
SGR (%/day)	1.11(±0.04)bc	1.08(±0.03)c	1.31(±0.09)a	1.19(±0.01)b	**
FCR	2.99(±0.14)a	2.95(±0.12)a	2.38(±0.25)b	2.72(±0.05)a	**
FCE	0.34(±0.02)b	0.34(±0.01)b	0.42(±0.04)a	0.37(±0.01)b	**
PER	0.79 (±0.04)b	0.79 (±0.03)	0.94 (±0.09)a	0.85(±0.01)ab	**
Survival rate (%)	100(±0.00)	100(±0.00)	100(±0.00)	100(±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 ($p< 0.05$) as per Duncan Multiple Range Test (Duncan, 1955) [3]. NS=Not significant, *significant in 5%, **significant in 1% significance level.

3.11 Gut micro biota 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 are shown in Table 4.

Table 4: 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	$1.1\sim2.5\times10^5$	$1.0\sim3.0\times10^3$
Treatment 2	$4.7\sim6.8\times10^5$	$4.2\sim10.0\times10^3$
Treatment 3	$1.8\sim9.1\times10^5$	$0.82\sim32.0\times10^3$
Treatment 4	$3.3\sim7.2\times10^6$	$3.4\sim5.2\times10^4$

3.12 Water quality parameters

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 26.70-30.60^{0}C, 8.5-10.20 mg/L and 8.0-8.60, respectively (Table 5).

4. Discussion

4.1 Growth performance

In the present study the effect of different levels of wheat supplementation on the production of catla fish was studied. At the end of experiment the highest mean final weight (g), weight gain (g) and percent weight gain (%), FCR, specific growth rate and survival rate was observed.

4.2 Weight gain

In this experiment the significantly ($P< 0.01$) highest weight gain was observed in treatment 3 which contains 10% whole wheat flour in the diet composition and the significantly highest percent weight gain was also found in treatment 3 which was about 1.31 where the lowest weight gain was observed in treatment 2 which contains 5% whole wheat flour in the diet composition. Salim *et al*. (2009) [13] assessed growth performance and FCR of wheat bran, rice broken and blood meal were evaluated in hybrid fish *C. catla* x *L. rohita*. The fish gained higher body weight (1.60 ± 0.14g) on wheat bran, followed by rice broken (1.51 ± 0.07g) and blood meal (1.24 ± 0.09g). The body weight of fish on wheat bran and rice broken was significantly higher ($p< 0.05$) than those fed blood meal, while the difference between the former two groups was non-significant. The percent weight gain in the present study was lower due to the quality of the fingerlings.

4.3 Specific growth rate

The specific growth rate (%/day) of *C. catla* under different treatments varied from 1.08 to 1.31. The significantly ($P< 0.05$) highest specific growth rate (%/day) was found at 10% whole wheat flour containing diet and nearly highest growth performance was found at 15% wheat flour containing diets. Haque (2006) [6] who recorded that mean SGR value was 1.44±0.52 in rohu monoculture system and this was higher than the mean SGR value of the present study due to the quality of the fingerlings.

4.4 Feed conversion ratio

Mean feed conversion ratio (FCR) in different treatments ranged from 2.38±0.25 to 2.99±0.14. The highest FCR was obtained in T_1 followed by T_2, T_4 and T_3, respectively. The significantly ($p< 0.05$) lowest FCR was found in T_3 and there was no significant ($P>0.05$) variation in mean FCR among T_1, T_2 and T_4 treatments. Srivastava *et al*. (2013) [14] assessed the effect of various types of feed ingredients on the biomass conversion rate to evaluate the use of agro-based products, as locally available feed ingredient materials for fish catla (*C. catla*) fingerling (av. wt. 1.52+0.11 to 1.55+0.07 g) growth performances. The FCR ranged between 2.34±0.11 to 2.98±0.09. In the present study the FCR ranged between 2.38±0.25 to 2.99±0.14 which indicates that it was very nearer to the previous study.

4.5 Survival rate (%)

In this experiment the survival rate of *C. catla* in different treatments was 100%. Yogesh *et al*. (2016) [15] evaluated the utilization of vegetables and fruits processing wastes as feed ingredient in the diet of *C. catla* fingerlings. Survival rate was good in all the treatments with mean survival value ranging from 83.33% to 100%. Mondal *et al*. (2010) [10] obtained survival of Tilapia in open ponds was also high rearing from 94.00% to 96.67%. No mortality was observed during the experimental period indicates that the water quality parameters were within suitable range for growth and survival of this species and fingerlings were maintained accurately.

4.6 Gut microbiota of *C. catla*

In this experiment the gut microbiota of *C. catla* was measured for different treatments after rearing 63 days in aquaria. The highest bacterial colony range was found in treatment 4 and the lowest range in treatment 1. In case of TSA agar media the highest range was $3.3\sim7.2\times10^6$ where the lowest range was $1.1\sim2.5\times10^5$. In case of MRS agar media the highest range was $3.4\sim5.2\times10^4$ which treatment probably possess the best prebiotic activity from wheat flour where the lowest range was $1.0\sim3.0\times10^3$. Ringo *et al*. (2006) reported that substituting dextrin with 15% inulin reduced the bacterial population from 4.8×10^5 to 3.56×10^4 level in the hindgut of Arctic charr, however the composition of bacteria colonizing the hindgut of Arctic charr fed inulin were dominanted by Gram-positive bacteria of the genera *Staphylococcus*, *Streptococcus*, *Carnobacterium* and *Bacillus*. Hoseinifar *et al*. (2014) [7] reported that in case of Caspian white fish (*Rutilus frisii*) fry all xylo oligosaccharide (XOS) fed fish showed significantly higher total viable heterotrophic aerobic bacteria (TAC) compared control treatment (0% XOS) ($P< 0.05$) and the highest levels were observed in Caspian white fish fry fed 2 or 3% dietary XOS. Interestingly, while no autochthonous lactic acid bacteria (LAB) were isolated from intestinal microbiota of fish fed control or 1% XOS diet, there were 3.80 ± 0.28 log CFU g-1 and 4.22±0.17 log CFU g-1 autochthonous LAB in fry fed 2 and 3% dietary XOS, respectively.

4.7 Water quality parameters

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 ^{0}C. Boyd (1982) reported that the range of water temperature from 26.06 to 3l.97^{0}C is suitable for fish culture. The dissolved oxygen content from present experiment ranged from 8.5 to 10.20 mg/L. Hossain (2009) [8] obtained dissolved oxygen level 5.l to 8.7 mg/L in ponds behind the Faculty of Fisheries, BAU campus. The oxygen content found in the present experiment lied within productive range. During the study period the pH value was found within the range of 8.0 to 8.60. Hossain (2009) [8] measured the pH value in ponds ofAgro-3 Farm, Trishal, Mymensingh ranged from 7.54 to 8.3 and 7.72 to 8.03. So the water quality parameters found in the present experiment lied within productive range.

The present study shows significant role of wheat supplementation on the growth performance of *C. catla* and the optimum inclusion level is 10%. So it can be concluded that 10% whole wheat flour as prebiotic compound has maximum importance in enhancing the production of *C. catla* and recommended to incorporate with the feed.

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