Bangladesh J. Fish. (2019) **31**(2) : 221-227

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

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Abstract. The aim of the study was to evaluate the suitability and efficacy of potato as prebiotic compound on the growth performance and survival rate of*Catla catla*. The experiment was carried out under 4 different treatments (T_1, T_2, T_3 and T_4) each with 3 replications for 63 days. The diets of T1, T2, T3 and T4 contained 0%, 5%, 10%, and 15% potato respectively. 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%.The final weight (g), weight gain (g), food conversion ratio (FCR), specific growth rate (%/day) and protein efficiency ratio (PER) were compared among the four treatments. Highest weight gain and specific growth rate were observed in T4 and lowest in T1. Highest FCR (6.18 ± 0.10) was found in T_1 and the lowest FCR (3.59 ± 0.18) was found in T_4. Highest PER (0.90 ± 0.009) was found in T_4 followed by T_3, T_2 and the lowest PER was found in T_1 (0.55 ± 0.005). Maximum gut microbiota was found in T_4 (9.6×10^7)and T_2 (7.5×10^4) in case of TSA agar media and CFU/ml, respectively.The best growth performance of *C. catla* was obtained from 15% potato containing diet. The study suggests that potato may be a suitable prebiotic for *C. catla*.
Key words: Prebiotic, FCR, PER, gut microbiota,*Catla catla*.

Introduction

The major carps are the most preferred farm fishes because of their fast growth and higher acceptability to consumers (Saini *et al*. 2014). Catla (*Catla catla*), known as the major (Indian) carp, is one of the most important aquaculture freshwater species in South Asian country. It is a highly growing species.Forcommercial fish farming; they can be fed on both natural and supplementary feeds and grow best at water temperature between 25°-32°C. Commercial carp fish feeds are usually good for them for better production. Fish feed is the most expensive input in aquaculture operations. Potato is best known for its carbohydrate content (approximately 26 gin a medium size potato). A medium-size potato with the skin provides 27 mg of vitamin C, 620 mg of potassium, 0.2 mg vitamin B_6 and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron, and zinc.The term potato as prebiotic compound is used to increase the fish production by enhancing growth rate of fish.

Prebiotics are carbohydrates that are not digested by fish, most of which are short chain monosaccharides commonly known as oligosaccharides. Prebiotics only promote the growth of beneficial microflora and/or reduce the growth of pathogenic microflora in the intestines of the host.Beneficial effect of prebiotics in fish include improved growth rate, feed efficiency, feed digestibility, survival, immunological status and resistance to bacterial and viral diseases, mainly due to modulation of the intestinal microbiota (Merrifield *et al*. 2010, Ringø *et al*. 2010, Dimitroglou *et al*. 2011). Therefore the present study was designed to evaluate the suitability and efficacy of potato as prebiotic compound on the growth performance and survival rate of *Catla catla*.

Materials and Methods

***Experimental design*:** The experiment was conducted in 12 glass aquaria (60L capacity) considering four treatments. Treatment 1 (T_1),Treatment 2 (T_2), Treatment 3 (T_3), and treatment 4 (T_4) each having three replications. The diets for T_1, T_2, T_3, T_4 contained 0%Potato, 5% Potato, 10% Potato and 15% Potato, respectively. *C. catla* fingerlings having an initial weight of 7.5 g were randomly distributed at a rate of 12 fish per aquaria. Sufficient level of dissolved oxygen in each aquarium was maintained with artificial aeration during the experimental period. The water temperature in the experimental aquaria ranged from 27.6-29.63°C. All the aquaria were kept on 1m high wooden table to facilitate better observation and accessibility. About 30% of water from each aquarium was changed daily during the removal of uneaten feed and faces.

***Sample collection and acclimatization*:** Fingerlings of *C. catla* were collected from Fisheries complex hatchery adjacent area of Bangladesh Agricultural University, Mymensingh. Transportation of fingerlings was done in oxygenated polyethylene bag to avoid stress and injury. During three days acclimation period, adequate oxygen supply was maintained through artificial aeration and fish were feed formulated pelleted feed.

***Feed formulation*:** The basal experimental diet wasformulated with the commonly available ingredients aspresented in Table I.The proximate compositions of the commonly available igredients in the experimental diets are presented in Table II and the proximate compositions of the formulated diet are presented in Table III. Four graded levels of potato 0%, 5%, 10% and 15% were included in the basal diet. The ingredients were ground, weighed, mixed, made dough of feed ingredients and pelleted with meat mincer through a 0.5 mm diameter. After pelleting, the feed were air dried and put in an air-tight container and stored at -20°C until fed.

Table I. Formulation of the basal diet (dry matter basis)

Ingredients	Diet 1	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
Total	100	100	100	100

***Proximate composition analysis*:** Proximate composition ofdifferent feed ingredients and prepared feeds were analyzed following Association of Officials Analytical Chemists (AOAC 2000) methods.

Table II. 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
Wheat bran	16.13	5.60	13.14	13.40	6.95	44.78
Soyabean oil	32.34	4.60	13.48	8.40	6.88	34.3

Table III. Proximate composition analysis of formulated feed (dry basis)

Diet	Moisture (%)	Crude Lipid (%)	Crude protein (%)	Ash (%)	Crude fibre (%)	NFE
Diet 1 (Control)	9.15	8.90	37.39	15.69	4.40	24.47
Diet 2	10.05	8.20	38.41	15.12	5.20	23.02
Diet 3	7.79	7.20	38.74	16.99	4.60	23.68
Diet 4	8.53	7.88	38.66	14.77	5.65	22.72

***Experimental procedure*:** The experiment was conducted for 63 days (from 16 August to 19 October, 2018) having three replications for each treatment. Initial weight of *C. catla* was 7.5g. Feeding was done twice daily at 9.00 am and 5.00 pm at the rate of 5% of the body weight throughout the study period. About 30% water was changed daily at 9.00 am. Initial and final weight of fish in each aquarium was recorded. Fish weight was measured at every seven days interval to keep record. Fish were caught by using a fine mesh scoop net and excess water then removed from fish body gently by using a blotting paper before weighing to the digital balance. After weighing the fingerlings were released in the aquarium.

***Estimation of growth performance*:** At the end of the experiment the growth performance of *C. catla* in terms of weight gain (g), specific growth rate (%/day), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE) and Protein Efficiency Ratio (PER)were calculated.

***Water quality parameters*:** The water quality parameters such as water temperature, dissolved oxygen (DO), pH were monitored biweekly throughout the study period to evaluate the environmental quality of aquaria.

***Isolation and enumeration of microbiota*:** Prebiotic treated *C.catla* was randomly treated (fish/treatment) for analyzing gut content of fish at the end of the experiment. After disinfection of the body surface of fish with 70% ethanol soaked cotton, the entire gut was carefully removed by using sterile forceps, 0.30 g to 0.40 g gut from each fish was cut by using sterile scissors and also homogenized by using 0.85% sterile physiological saline at 4°C. Resultant aliquot was serially diluted, plated on Trypton Soya agar (TSA) and De Rogosa and Sharp agar (MRS) media was incubated for 24hrs at 37°C to recover bacteria of gut samples. The bacterial colony of gut samples were expressed as Number of colony forming Units/ml (CFU/ml).

***Data analysis*:** The collected data were subjected to a one way analysis of variance (ANOVA) by Microsoft Excel and XL-Stat (Ver. 2013). The level of significance was set at $p < 0.05$ to see whether the influence of different treatments on these parameters were significant or not. The means of different treatment were compared by Duncan Multiple Range Test (Duncan 1955) to examinethe significance of variation between the treatment means.The graphs were made using MS Excel.

Results

***Weight gain (g)*:** The mean final weight of individual *C. catla* in different treatments varied from 10.61 g to 13.28 g. The mean weight gain (g) in treatment T_4 was found to be the highest followed by T_3, T_2 and T_1, respectively (Table IV). 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 T_4 followed by T_3, T_2 and T_1 respectively (Table IV).

***Specific growth rate (%/day)-SGR*:** The specific growth rate (%/day) ranged from 0.55% to 0.91% /day. The highest specific growth rate (0.91%/day) was found in treatment T_4 followed by T_3, T_2 T_1, respectively. There was significance ($p < 0.05$) vardifference in SGR between T_1 and T_3; T_1 and T_4 but no significance difference was observed between T_2 and T_1; T_2 and T_3; T_3 and T_4 (Table IV).

***Food conversion ratio (FCR)*:** Mean FCR in different treatments ranged from 3.59 to 6.18. The highest FCR was obtained in treatment T_1 followed by T_4, T_3, and T_2, respectively. There was no significant ($p > 0.05$) variation in mean food conversion ratio (FCR) amongT_2, T_3andT_4 but has significant difference($p < 0.05$) between T_1 and other treatments (Table IV).

Food conversion efficiency (FCE): Mean FCE in different treatments ranged from 0.12 to 0.20 (Table IV).The highest FCE was obtained in T_4followed by T_3, T_1, and T_2, respectively. There was no significant ($p \geq 0.05$) variation in mean food conversion efficiency (FCE) among T_2, T_3 and T_4 but has significant variation ($p < 0.05$) between T_1 and others (Table IV).

***Protein efficiency ratio (PER)*:** Mean PER in different treatments varied from 0.55 to 0.90 (Table IV). The highest protein efficiency ratio (PER) was found in treatment T_4 followed by T_3, $T_2$and T_1, respectively. There was significance ($p < 0.05$) difference between T_1 and T_4; T_2 and T_4 but no significance variation between T_3 and T_2; T_3 and T_4; T_3 and T_1 and T_2 ($p > 0.05$) (Table IV).

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

Table IV. The effect of different treatments on growth performance, feed utilization and survival of (*Catla catla*) reared in aquarium (Mean±SE) during the study period

Variable parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	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	**
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)	100 (±0.00)	100 (±0.00)	100 (±0.00)	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). NS= Not significant, * = significant in 5%, ** = significant in 1% significance level.

***Gut microbiota in TSA and MRS agar media in different treatments*:** 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 levelof gut microbiota (9.6×10^7CFU/ml) was found in T_4 that contained 15% potato followed by T_2, T_3 and T_1, respectively (Table V) and in case of MRS agar media the highest levelwas found in T_2 (7.5×10^4 CFU/ml) which contained 5% potato followed by T_4, T_2 and T_1, respectively (Table V). The lowest bacterial load was found in treatment 1 under controlled condition which contained 0% potato in diet composition.

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

Treatments	TSA agar media (CFU/ml)	MRS agar media (CFU/ml)
Treatment 1	1.7×10^6	5.7×10^2
Treatment 2	1.2×10^7	7.5×10^4
Treatment 3	1.1×10^7	1.22×10^3
Treatment 4	9.6×10^7	3.03×10^3

Water quality parameter: The good water quality is very essential for survival and growth. The water quality parameters such as temperature, dissolved oxygen and pH of different tank were measured throughout the study period. The range of temperature, dissolved oxygen and pH were 26.70-30.60°C, 8.5-10.20 mg/l and 8.0-8.60, respectively (Table VI).

Discussion

In this study, the effect of different levels of potato on growth performance of *C. catla* in aquarium was evaluated. 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, without potato supplement (control). 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 $(0.55\pm0.03)\%$ to $(0.91\pm0.03)\%$. Popma (1982) 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. 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 T_4 (15% potato). 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* x *Labeo rohita*). 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*. Mamun *et. al* (2014) observed that the survival rate of catla x rohu, catla x mrigal and mrigal x rohu were 79.01, 69.14 and 46.15%, respectively.

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 7.5×10^4CFU/ml in treatment 2 (5% potato) and lowest was 5.7×10^2CFU/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. These values are low compared to autochthonous levels previously reported in Atlantic salmon (Merrifield *et al.* 2009). Salinas *et al.* (2008) exposed to *Carnobacterium 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 10^4-10^6 CFU/g intestines 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. In the present study the water temperature 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 (2004) measured the water temperature in ponds of BAU campus, Mymensingh ranged from 29.4 to 33.0°C and 26.0 to 32.8^0C, 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. In this experiment pH value was recorded 7.98 to 8.20. The recorded parameters in the experiments were within the optimum range for fingerlings rearing.

Acknowledgement: The authors are grateful toNational Agricultural Technology Program (NATP) for funding to support this work.

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(Manuscript Received 17 November 2019)