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RECENT TRENDS IN ENVIRONMENTAL SUSTAINABILITY

Effect of eco-friendly probiotics-supplemented rapeseed meal–based diet on the performance of *Catla catla* fingerlings

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Abstract

Ever-increasing human population compels the researchers to search for alternative food sources such as fish meat. For increase of fish growth and proper feed utilization, probiotics were added in rapeseed meal-based diet in current trial for proper digestion and absorption of nutrients in fish and ultimately higher growth with lower aquatic pollution. Fish gut microbiota is important for the feed utilization and absorption in body for higher growth. A 70-day study was conducted to investigate the effects of probiotics-supplemented rapeseed meal-based diet on growth performance, digestibility of nutrients, and absorption of minerals in *Catla catla* fingerlings with lowering water pollution level. Six test diets were prepared by using different levels of multi-strain probiotics i.e. 0, 1, 2, 3, 4 and 5 g/kg (0.0–0.5%) in rapeseed meal-based diet. At the rate of 4% of live wet weight, *Catla* fingerlings were fed two times a day and faeces samples from each tank were collected. According to the results, it was observed that probiotics supplementation (@2 g/kg) in rapeseed meal-based diet resulted with improvement in nutrient digestibility (CP, 72%; fat, 75% and GE, 70%), mineral absorption (Ca, 72%; Na, 76%; K, 70% and P, 70%), specific growth rate (SGR, 1.55), improved feed conversion ratio (FCR, 1.22) and weight gain percentage (WG%, 303%) of fingerlings. It was also noticed that probiotics supplementation decreased the discharge of minerals and nutrient through faeces, as compared to control diet; hence, it plays a significant role in reducing water pollution. On the basis of these results, it was concluded that probiotics inclusion at level of 2 g/kg was useful to formulate the cost effective and eco-friendly fish feed with the maximum improvement in growth and fish health by using rapeseed meal-based diet, as compared to control and other test diets.

Keywords Rapeseed meal · Probiotics · *C. catla* · Growth performance · Nutrient digestibility · Mineral absorption

Introduction

The major carp calta (Thaila) belongs to a family Cyprinidae and is a most important commercial fish with utmost market interest due to its taste and flesh (Renuka et al. 2014). It is native to rivers and lakes of Pakistan, Bangladesh, northern India, Myanmar and Nepal. Approximately 12% of human food is provided by the aquaculture industry. From the past few decades, the aquaculture industry is flourishing rapidly in Asia. In Pakistan, aquaculture is in progress, and increasing needs must be fulfilled for development of the existing fish culture potential. Aqua farming supports the world's economy and fulfill the increasing demand for seafood globally with the purpose of producing food source (Iqbal et al. 2015; Abbas et al. 2019). Fish has an excellent protein quality and safe source for better human health (FAO 2018; Van Doan et al. 2019). As fish consumption and world population

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growth increase massively, global fish demand has been increased (Carbone and Faggio 2016).

In the perspective of the fast increase in the rearing of aquatic animals, there is a worldwide search for low price and nutritionally balanced ingredients for the manufacturing of commercial feed to satisfy this increasing demand for the aquaculture sector (FAO 2016). Widely used in fish feed is the fish meal (FM) which is a best protein source, an incredibly nutritious and adequately acceptable source of protein, but the high cost of FM is being driven by its increasing demand (Mohapatra et al. 2012). Research was therefore conducted in order to search the appropriate sources of protein to lessen their cost of feed production and sustain their affordability (Hardy 2010; Shahzad et al. 2021). Some researchers have clearly mentioned the affordability of the plant-based protein components, as they are of low cost than fish meal and easily available throughout the year (Bonaldo et al. 2011; Hussain et al. 2018). Plant-based proteins such as soybean meal, wheat middlings, pea, sunflower meal, linseed, moringa, corn gluten and rice meal are typically used to minimize fish meal intake in feed (Song et al. 2014; Newaj-Fyzul and Austin 2015; Dawood et al. 2017). The by-product of canola oil production from the rapeseed plant is rapeseed meal (RM), which is currently being used as an alternative source of protein for Thaila fingerlings.

RM contains high contents of protein, as compared to other plant protein sources (Burel et al. 2000), unsaturated fats, favourable profile of peptides, microelements, minerals and other vitamins (Luo et al. 2012). It was showed that RM proves a better substitute of FM without having any adverse effects on development and use of feed (Bjornsdottir et al. 2010). It also proves itself a potential feed source and could reduce costs in aquaculture as a substitute for FM (Bu et al. 2018). However, addition of RM in the diet would reduce fish development and protein effectiveness ratio due to lower microbiota in the digestive system for proper digestibility and absorption (Tan et al. 2013; Butt and Volkoff 2019). Fish gut microbes are most often known as an "extra organ" because it performs a pivotal role in the development and functions of the intestines as well as its overall development, mineral and nutrient intake, maturation and fitness (Abarike et al. 2018; Feng et al. 2018).

Probiotics are living bacteria that induce health advantages by improving gut activity when added in appropriate quantities (FAO/WHO 2002). Many probiotics are currently used in plant-based protein diets including Lactobacillus, Enterococcus, Bacillus, Clostridium and a number of other related groups (Nayak 2010; Shahzad et al. 2021). Improving water quality and promoting host species nutrition can also be achieved by producing additional digestive enzymes by probiotics (Verschuere et al. 2000). They increase the capacity of the intestinal surface absorption (Pirarat et al. 2011) and absorptive potential of microvilli (Standen et al. 2015).

The use of probiotics as feed additives is essential to enhance nutrient usage, feed quality and growth (Standen et al. 2016). When digestive system loses good bacteria, probiotics utilization in feed helps to improve the performance of fish by improving the digestibility of nutrients (Lara-Flores et al. 2003), improve feed utilization and weight gain due to probiotics (Ramos et al. 2017; Singh et al. 2017; Hussain et al. 2018). It has been observed that probiotics supplementation has an important role in enhancing mineral absorption in fish body when fish fed plant meal-based diet (Hussain et al. 2018). The present study was designed to check the impacts of probiotics supplemented rapeseed meal-based diet especially in terms of improving growth, digestibility of nutrients, retention of minerals in *Catla catla* fingerlings and for the production of low cost and eco-friendly feed for fish.

The key determinants impacting nutritional loading by fish are the nutrient content and composition of the diet (Cho and Bureau 2001), hence dietary practises determine the magnitude of the environmental influence (Berzi-Nagy et al. 2021). Plant meal-based diets along with supplements like probiotics improves of ingestion of feed and protects the environment from polluting.

Materials and methods

The research was conducted to find out the effects of rapeseed meal-based diets supplemented with probiotics on nutrient digestibility, mineral absorption and growth of *C. catla* fingerlings in the Fish Nutrition Lab, Zoology Department, University of Education, Lahore.

Experimental conditions and fish

After collecting from hatchery, saline solution (0.5%) was prepared to treat *C. catla* fingerlings for 1 to 2 min for the removal of pathogens (Rowland and Ingram 1991), and they were acclimatized to laboratory conditions for 14 days before the start of the experiment. Fingerlings were placed in V-shaped water tanks with a capacity of approximately 70 L water. The fingerlings were fed twice a day on their respective diets (Allan and Rowland 1992). There was regular monitoring of important water quality parameters i.e. temperature (24.9–28.7 °C), pH (7.4–8.6) and dissolved oxygen (5.8–7.3 mg/L). The air was provided throughout the experiment by a capillary system using an air pump.

Experimental design

Rapeseed meal was used for the preparation of six test diets with different concentrations of commercial probiotics at the rate of 0, 1, 2, 3, 4 and 5 g/kg (0.1–0.5%); CFU $g^{-1} = 2 \times 10^9$. Total duration of experimental trial was of 70 days. For bacterial viability and count in all diets, LIVE/DEAD *Bac*Light viability staining and CSLM enumeration method were used (Auty et al. 2001). One control and five test diets containing probiotics were fed to six groups of fish. Each group of fifteen fish was placed in triplicate water tanks. Fingerlings were fed at the rate of 4% live body weight. Each rapeseed meal-based diet supplemented with probiotics was compared with other diets and control diet to determine growth performance parameters, nutrient digestibility and mineral absorption by using completely randomized design (CRD).

Feed pellet formation

Rapeseed meal and experimental feed ingredients (including required quantity of probiotics for each test diet) were purchased from a local market and were analysed for chemical composition by globally recognized methods of AOAC (1995) before the experimental feed formulation. All components including fish oil (Table 1) were blended for 10 min in an electric mixer. Prior to the preparation of pellets by a pelleting machine, dough was made by addition of water. All formulated diets except control diet were supplemented with the graded levels of probiotics, as mentioned earlier. All experimented diets were dried under a shady place and preserved at 4 °C before use in air-tight jars.

Protocol of feeding and sample collection

After 2 h of feeding session, left-over feed was collected from every tank through a collection tube of tanks, and tanks were properly washed before the refilling process. The faecal material was collected after 2 h very carefully to prevent the discharge of nutrients in water. The faecal material was dried at 65 °C in an oven till complete de-moisturization and samples were stored at room temperature for further analysis.

Estimation of nutrient digestibility

Following a 70-day feeding trial, homogenized feed and faeces samples were oven-dried for 12 h at approximately 105 °C for calculation of moisture content of sample diets and faeces. A micro Kjeldahl Apparatus was utilized for determination of crude protein (CP) contents ($N \times 6.25$), while crude fat was analysed using the Soxhlet extraction apparatus by ether extraction method. The crude fibre contents were estimated as a drop on the combustion of dried lipid-free materials after treating with 1.25% sulphuric acid and 1.25% sodium hydroxide. Using standard formula, total carbohydrates in feed and faeces (N-free extract) were estimated i.e.

$$\text{Total carbohydrates } (\%) = 100 - (EE\% + CP\% + \text{Ash}\% + CF\%).$$

To analyse the GE, an oxygen bomb calorimeter was used.

Table 1 Diet ingredients and composition on dry basis

Ingredients	Test diet composition	Chemical composition (%) of fish feed ingredients						
		Dry matter (%)	Gross energy (kcal/g)	Crude protein (%)	Ether extraction (%)	Crude fibre (%)	Ash (%)	Carbohydrates (%)
Rapeseed meal	34	92.87	3.73	33.67	6.72	2.64	6.39	50.58
Wheat flour*	12	91.63	2.69	9.78	2.18	2.79	3.32	81.93
Soybean	16	91.84	4.23	32.54	5.96	2.41	2.31	56.78
Rice polish	12	93.71	4.19	13.67	10.17	3.78	6.91	65.47
Fish meal	14	92.07	3.81	44.92	8.62	1.59	21.45	23.42
Fish oil	8							
Vitamin premix**	1							
Chromic oxide	1							
Mineral premix***	1							
Ascorbic acid	1							
Probiotics****	0–5 g/kg (0.0–0.5%)							

*Probiotics were used at the expense of wheat flour. **Vitamin D_3 (3,000,000 IU), vitamin E (30,000 IU), vitamin A (15,000,000 IU), vitamin B_1 (3000 mg), vitamin B_2 (7000 mg), vitamin B_6 (4000 mg), vitamin B_{12} (40 mg), vitamin C (15,000 mg), vitamin K_3 (8000 mg), nicotinic acid (60,000 mg), calcium pantothenate (12,000 mg) and folic acid (1500 mg). ****P* (135 g), Na (45 g), Fe (1000 mg), Ca (155 g), Mg (55 g), Cu (600 mg). *****Lactobacillus bulgaricus*, *L. acidophilus*, *Bifidobacterium lactis*, *L. rhamnosus*, *B. bifidum*, *Bifidobacterium*, *Streptococcus thermophiles*

Study of growth

Fifteen fingerlings with an average initial weight (7 g) and length (14 cm) were stocked in each tank. For the evaluation of *Catla* fingerlings' growth, weight was noted from each tank on fortnightly basis throughout the experimental period. Standard formula (NRC 1993) was computed to calculate the parameters such as SGR, weight gain (g), feed conversion ratio (FCR) and weight gain (percentage).

$$\text{Weight gain } \% = \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100 \tag{1}$$

$$FCR = \frac{\text{total dry feed intake (g)}}{\text{wet weight gain (g)}} \tag{2}$$

$$SGR\% = \frac{(\text{ln. final wt. of fish} - \text{ ln. initial wt. of fish})}{\text{trial day}} \times 100 \tag{3}$$

Mineral estimation

The minerals (Ca, Fe, Al, Mg and Cu) were estimated after adequate, dilution according to AOAC (1995) by using an atomic absorption spectrophotometer. A flame photometer was used in analysis to estimate sodium and potassium. Using the UV–VIS 2001 spectrophotometer at absorption rate of about 370 nm, the contents of Cr_2O_3 were estimated after oxidation with molybdate reagent in faeces and diet samples (Divakaran et al. 2002). A spectrophotometer (UV–VIS Spectrophometer) with an absorption frequency of approximately 720 nm was used to analyse the phosphorous concentration of experimental feed and faecal matter.

Calculation of ADC%

The standard formula was used to calculate the apparent digestibility coefficients (percentage) in formulated test diets (NRC 1993).

$$ADC\ (\%) = 100 - 100 \times \frac{\%\text{ marker in diet } \times \ \%\text{ nutrient in faeces}}{\%\text{ marker in faeces } \times \ \%\text{ nutrient in diet}} \tag{4}$$

Statistical analysis

Furthermore, data on ADC% of nutrients and minerals in body and growth were subjected to one-way ANOVA and polynomial orthogonal contrast. SPSS Computer Package was used to find the means that were significantly different ($p < 0.05$) in terms of combined, linear and quadratic analyses from each other (Table 2).

Table 2 Ingredients composition (%) of rapeseed meal meal-based test diets supplemented with probiotics and chemical composition of feed ingredients

Diets	Probiotics level (g/kg)	CP in diet	Fat in diet	GE in diet
TD-I (control diet)	0	31.09 ± 0.23	6.73 ± 0.17	3.79 ± 0.14
TD-II	1	31.05 ± 0.17	6.73 ± 0.13	3.80 ± 0.18
TD-III	2	30.88 ± 0.60	6.72 ± 0.19	3.81 ± 0.16
TD-IV	3	31.13 ± 0.23	6.74 ± 0.13	3.80 ± 0.17
TD-V	4	31.12 ± 0.18	6.75 ± 0.19	3.79 ± 0.11
TD-VI	5	30.77 ± 0.61	6.74 ± 0.18	3.81 ± 0.11

Data in each is mean of triplicates ($n = 3$)

Results

Nutrient digestibility parameters

In the current research study, it has been noted that all the diets prepared by using rapeseed meal by-products contain an equal amount of nutrients i.e. EE, CP and GE. The results of analysed nutrients (EE, CP and GE) in faeces and digestibility in fish are shown in Table 3.

Analysed constitution of nutrients in defecated matter by *C. catla* fingerlings was statistically different ($p < 0.05$) when fingerlings were given probiotics including RSM-based diet. The results have shown that there was a lowest discharge of nutrients in faeces, and maximum nutrients were retained in the fish body, as fingerlings were given 2 g/kg probiotics supplemented rapeseed meal-based diet followed by 3 g/kg level-based diet. However, maximum nutrient release via faeces occurred when fish were fed on diet without probiotics inclusion (0 g/kg). Additionally, it was observed that fish fed on probiotics supplemented rapeseed meal-based diets altogether showed better ADC% of nutrients in comparison to non-substituted diet. The ADC% values were increased with a surge in probiotics level at 3 g/kg level-based diet and were found maximum at 2 g/kg diet. It was observed that percentage of ADC of nutrients could not improve on further supplementary addition of probiotics (i.e.: 4 g/kg and 5 g/kg). Highest values of digestibility of protein (72%), fat (75%) and GE (69%) were noted at 2 g/kg level which was significantly different from other test diets. Second higher values (CP, 68%; fat, 70% and GE, 68%) were obtained at 3 g/kg supplemented level of probiotics which were significantly different with each other ($p < 0.05$). These mean values were statistically maximum ($p < 0.05$) in comparison to non-substituted and other formulated test diets. Fish fed with test diet-VI (5 g/kg) indicated lowest digestibility of protein (57%), lowest digestibility of GE (57%) and fats (56%) were found in group I (1 g/kg level) and in control group, subsequently. In polynomial orthogonal contrast analysis,

Table 3 Analysed composition of nutrients in feces and apparent digestibility coefficient percentage of CP, fat and GE in *C. catla*

Diets	Probiotics level g/kg	CP in faeces	CP digestibility	Fat in faeces	Fat digestibility	GE in faeces	GE digestibility
TD-I (control diet)	0	13.94 ± 0.35^{a}	57.50 ± 0.58^{e}	3.09 ± 0.15^{a}	56.48 ± 0.63^{d}	1.70 ± 0.11^{a}	57.63 ± 0.75^{d}
TD-II	1	11.39 ± 0.48^{b}	65.39 ± 0.85^{c}	2.25 ± 0.06^{bc}	68.41 ± 0.71^{b}	1.74 ± 0.07^{a}	56.81 ± 0.54^{d}
TD-III	2	9.03 ± 0.34^{d}	72.34 ± 0.81^{a}	1.80 ± 0.12^{d}	74.72 ± 0.94^{a}	1.23 ± 0.07^{b}	69.48 ± 0.97^{a}
TD-IV	3	10.37 ± 0.30^{c}	68.34 ± 0.90^{b}	2.09 ± 0.07^{cd}	70.48 ± 0.97^{b}	1.26 ± 0.08^{b}	68.46 ± 0.71^{a}
TD-V	4	12.24 ± 0.25^{b}	62.64 ± 0.64^{d}	2.52 ± 0.10^{b}	64.47 ± 0.90^{c}	1.37 ± 0.07^{b}	65.59 ± 0.96^{b}
TD-VI	5	14.23 ± 0.37^{a}	56.49 ± 0.67^{e}	2.98 ± 0.16^{a}	58.37 ± 0.94^{d}	1.60 ± 0.07^{a}	60.50 ± 0.53^{c}
p-Value	Combined	.000	.000	.000	.000	.000	.000
	Linear	.009	.000	.321	.138	.002	.000
	Quadratic	.000	.000	.000	.000	.000	.000

Means in columns possessing dissimilar superscripts are statistically dissimilar at $p \leq 0.05$; data in each is mean of triplicates ($n = 3$)

CP crude protein, *GE* gross energy

it was found that nutrient in faeces and digestibility values were significantly different from each other in combined and quadratic way but were a little bit similar in linear term, as shown in Table 3. From these analyses, it was noted that the digestibility values of each test diet by fish were different from each other which shows a statistical difference in nutrient discharge through faeces. These values provide a convincing evidence that minimum nutrient release resulted in higher ADC% with reference to nutrients in fingerlings when given rapeseed meal-based diet supplemented with probiotics. The current study distinctly indicated that probiotics supplementation at 2 g/kg is optimum for paramount nutrient retention in Thaila fingerlings protecting the water body from polluting and clean environment by digesting nutrients.

Mineral absorption

Analysis showed equal mineral values in all test diets and control diet (Table 4). It was observed that faeces of fish fed on various levels of probiotics included diet containing different levels of mineral, as compared to their respective diets (Table 5). Al was barely detected in test diets as its amount was minimum in diets. Minimum release of minerals (P, Na, K, Ca, Fe, Al, Mg and Cu) was observed at 2 g/kg level followed by 3-g/kg supplemented diet during the whole research period. Analysis showed that discharge of minerals by Thaila fed on probiotics-supplemented diets was consistently lower than fish fed on control diet (Table 5). It has been observed that the further increase in probiotics level lessens the uptake of minerals. Table 6 shows that minerals like P (69%), Na (75%), K (70%) and Ca (72%) had the most significant absorption ($p < 0.05$) at 2 g/kg probiotics supplemented in rapeseed meal-based diet. While other minerals such as Fe (67%), Mg (63%) and Cu (62%) had greater absorption at 4 g/kg level of probiotics in rapeseed meal-based diets, Al (66%) showed maximum absorption at 5 g/kg level.

Lowest absorption of minerals such as P (53%), Na (55%), K (58%), Ca (53), Fe (50%) and Mg (53%) was noted in fish fed on control diet. Minerals like Al (55%) and Cu (52) showed lowest absorption at 1 g/kg level. It was noted that from 2 to 4 g/kg level of probiotics supplementation in rapeseed meal-based diet indicated maximum absorption

Table 4 Percentage composition of minerals in control and test diets based on rapeseed meal

Minerals	TD-I (control) Probiotics levels (g/kg)	TD-II	TD-III	TD-IV	TD-V	TD-VI
	0	1	2	3	4	5
Fe	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Ca	0.47 ± 0.03	0.41 ± 0.01	0.28 ± 0.03	0.33 ± 0.03	0.38 ± 0.02	0.45 ± 0.04
P	2.10 ± 0.11	2.10 ± 0.09	2.10 ± 0.13	2.11 ± 0.10	2.09 ± 0.11	2.09 ± 0.12
Na	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Al	0.001 ± 0.0001	0.001 ± 0.0001	0.001 ± 0.0001	0.001 ± 0.0001	0.001 ± 0.0001	0.001 ± 0.0001
K	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Mg	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Cu	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Data in each is mean of triplicates ($n = 3$)

Table 5 Percentage composition of minerals in feces of *C. catla* (fingerlings) fed probotics-supplemented rapeseed meal-based diets

Minerals	TD-I (control) Probiotics level (g/kg)	TD-II	TD-III	TD-IV	TD-V	TD-VI
	0	1	2	3	4	5
P	1.03 ± 0.05^{a}	0.90 ± 0.02^{b}	0.68 ± 0.03^{d}	0.73 ± 0.04^{d}	0.76 ± 0.06^{cd}	0.86 ± 0.03^{bc}
Na	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
K	0.64 ± 0.02^{a}	0.56 ± 0.02^{bc}	0.44 ± 0.03^{e}	0.47 ± 0.03^{de}	0.52 ± 0.02^{cd}	0.61 ± 0.02^{ab}
Ca	0.43 ± 0.01^{a}	0.37 ± 0.04^{ab}	0.25 ± 0.00^{d}	0.30 ± 0.02^{cd}	0.34 ± 0.03^{bc}	0.41 ± 0.03^{ab}
Fe	0.03 ± 0.00^{a}	0.03 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.02 ± 0.00^{b}	0.02 ± 0.00^{ab}
Al	0.0003 ± 0.00004^{a}	0.0003 ± 0.00004^{a}	0.0003 ± 0.00003^{a}	0.0002 ± 0.00004^{a}	0.0002 ± 0.00004^{a}	0.0002 ± 0.00004^{a}
Mg	0.005 ± 0.0001^{a}	0.005 ± 0.0002^{a}	0.004 ± 0.0002^{ab}	0.004 ± 0.0003^{bc}	0.004 ± 0.0003^{c}	0.004 ± 0.0002^{abc}
Cu	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means in rows ($n=3$) possessing dissimilar superscripts are statistically dissimilar at $p<0.05$

Table 6 Percentage composition of minerals absorption in *C. catla* (fingerlings) fed probiotics-supplemented rapeseed meal-based diets

Minerals	TD-I (control) Probiotics level (g/kg)	TD-II	TD-III	TD-IV	TD-V	TD-VI	*p*-Value		
	0	1	2	3	4	5	*L*	*Q*	*C*
P	53.34 ± 0.80^{d}	59.42 ± 0.91^{c}	69.60 ± 0.78^{a}	67.22 ± 0.81^{ab}	65.43 ± 0.99^{b}	61.28 ± 0.90^{c}	.000	.000	.000
Na	55.46 ± 0.95^{d}	67.51 ± 0.85^{c}	75.56 ± 0.89^{a}	70.78 ± 0.89^{b}	66.57 ± 0.82^{c}	56.05 ± 0.94^{d}	.306	.000	.000
K	57.55 ± 0.95^{d}	62.45 ± 0.65^{c}	70.35 ± 0.81^{a}	68.77 ± 0.88^{a}	65.53 ± 0.97^{b}	59.45 ± 0.79^{d}	.001	.000	.000
Ca	52.52 ± 0.95^{f}	58.63 ± 0.64^{d}	71.56 ± 0.89^{a}	66.64 ± 0.80^{b}	61.60 ± 0.63^{c}	55.52 ± 0.88^{e}	.000	.000	.000
Fe	49.50 ± 0.61^{f}	54.65 ± 0.78^{e}	58.46 ± 0.70^{d}	64.38 ± 0.96^{b}	66.82 ± 0.92^{a}	61.55 ± 0.85^{c}	.000	.000	.000
Al	59.69 ± 0.84^{c}	55.30 ± 0.86^{d}	58.48 ± 0.88^{c}	63.41 ± 0.92^{b}	65.47 ± 0.87^{ab}	66.37 ± 0.56^{a}	.000	.000	.000
Mg	53.21 ± 0.84^{e}	54.44 ± 0.96^{de}	56.60 ± 0.71^{cd}	59.59 ± 0.98^{b}	62.67 ± 0.93^{a}	58.32 ± 0.91^{bc}	.000	.000	.000
Cu	54.40 ± 0.69^{cd}	52.39 ± 0.97^{d}	56.50 ± 0.85^{bc}	61.24 ± 0.94^{a}	62.41 ± 0.83^{a}	58.38 ± 0.96^{b}	.000	.000	.001

Means in rows ($n=3$) possessing dissimilar superscripts are statistically dissimilar at $p<0.05$

L linear, *Q* quadratic, *C* combined

in comparison with other diets having different probiotics inclusions and control diet (Table 6). Less release of minerals in water reduced water pollution and turbidity. That is why, this work is also helpful to minimize the level of water pollution and expenses of extra supplementation of minerals in diet that will reduce the overall feed cost. In statistical analysis with polynomial orthogonal contrast, all the minerals were significantly different from each in terms of combined, linear and quadratic analyses except sodium, that was statistically correlated with each other as given in Table 6.

Growth study

According to our study, most significant results were obtained over growth (FCR, SGR, weight gain and weight gain%) in *C. catla* fingerlings fed on probiotics-supplemented diet. Table 7 shows the average values of various growth performance parameters. The results showed that diet with the supplementation of probiotics-augmented growth performance when compared to diet without probiotics. Initial weight (approx. 6.7 g) was quite similar in all of the fish groups, whereas all the parameters were statistically different when they were analysed after the completion of the trial. Highest final weight (27.11 g), weight gain (20 g), weight gain percentage (303%), SGR (1.55) and survival rate (98%) were observed in fish fed on diet-III that was supplemented with 2 g/kg level of probiotics in rapeseed meal-based diet; whereas, the least value for FCR was also recorded in the same group of fish fed with same diet III (2 g/kg).

Lowest weight gain (14 g), weight gain percentage (191%), final weight (19 g), survival rate (91%) and SGR (1.19) were found at 5 g/kg level of probiotics supplemented in rapeseed meal-based diet. Final weight, weight gain and weight gain% were found to increase from 1 to 2 g/kg level. Decrease in growth was recorded when fish were fed with additional amount of probiotics i.e. 5 g/kg. The results indicated that *C. catla* feeding on diet supplemented with optimum level of 2 g/kg of probiotics considerably

Table 7 Growth performance of *C. catla* (fingerlings) fed probiotics-supplemented rapeseed meal-based diets

Diets	Probiot-icslevels g/kg	IW	FW	WG	WG%	WG (Fish^{-1} day^{-1}) g	Survival %	FCR	SGR
TD-I (control diet)	0	6.76 ± 0.18[a]	20.84 ± 0.68[de]	14.08 ± 0.50[de]	208.20 ± 2.27[cd]	0.16 ± 0.01[de]	93.33 ± 0.00[a]	1.56 ± 0.02[b]	1.25 ± 0.01[cd]
TD-II	1	6.74 ± 0.23[a]	23.69 ± 0.74[b]	16.95 ± 0.93[b]	251.99 ± 21.85[b]	0.19 ± 0.01[b]	95.56 ± 3.85[a]	1.33 ± 0.04[d]	1.40 ± 0.07[b]
TD-III	2	6.73 ± 0.19[a]	27.11 ± 0.71[a]	20.38 ± 0.68[a]	302.94 ± 13.37[a]	0.23 ± 0.01[a]	97.78 ± 3.85[a]	1.22 ± 0.03[e]	1.55 ± 0.04[a]
TD-IV	3	6.75 ± 0.14[a]	23.21 ± 0.84[bc]	16.46 ± 0.98[bc]	244.06 ± 19.44[bc]	0.18 ± 0.01[bc]	97.78 ± 3.85[a]	1.37 ± 0.03[d]	1.37 ± 0.06[bc]
TD-V	4	6.72 ± 0.26[a]	21.63 ± 0.66[cd]	14.91 ± 0.58[cd]	221.95 ± 11.21[bcd]	0.17 ± 0.01[cd]	95.56 ± 3.85[a]	1.45 ± 0.03[c]	1.30 ± 0.04[bcd]
TD-VI	5	6.72 ± 0.16[a]	19.59 ± 0.81[e]	12.87 ± 0.66[e]	191.54 ± 5.79[d]	0.14 ± 0.01[e]	91.11 ± 3.85[a]	1.64 ± 0.03[a]	1.19 ± 0.02[d]
p-Value	Combined	1.000	.000	.000	.000	.000	.223	.000	.000
	Linear	.797	.001	.001	.005	.001	.525	.000	.004
	Quadratic	1.000	.000	.000	.000	.000	.018	.000	.000

Means in rows ($n = 3$) possessing dissimilar superscripts are statistically dissimilar at $p < 0.05$

L linear, *Q* quadratic, *C* combined

stimulated growth rate ($p < 0.05$), as compared to diet without probiotics.

The highest level of probiotics inclusion (5 g/kg) and least level (0 g/kg) of probiotics indicated minor differences in values of growth parameters. In fish feeding on diet III (2 g/kg) of probiotics, the best value of FCR (1.22) was obtained accompanied by the diet II based on 1 g (1.33) of probiotics level. The poor value of FCR (1.64) was determined in diet VI (5 g/kg probiotics) followed by the control diet (1.56). Less FCR means the largest conversion of feed into the body mass of fish and minimum discharge of feed through faeces. Similarly, majority of growth indices (WG, FW, WG%, WG fish^{-1} day^{-1} g, FCR and SGR) were statistically different from each other in all (combined, linear and quadratic) tests. The results indicated that the optimum level of probiotics supplementation in fish feed for maximum growth is 2 g/kg. From these results, it could be inferred that probiotics inclusion in RSM-based diet is crucial for maximum growth, nutrient digestibility and mineral retention in fresh water *C. catla* fingerlings.

Discussion

Probiotics play a crucial role in enzyme functioning which results in better feed efficiency or also through alteration in gut environment which can enhance mineral absorption in *Catla* (Shelby et al. 2006; Ghosh et al. 2007; Pirarat et al. 2011; Tanaka et al. 2012). Due to sequestering and adhesion action of probiotics, better mineral absorption occurs in the fish body (Merrifield and Ringo 2014). Probiotics stimulate digestive enzyme production which leads to increased usage of ingested substances by increasing the absorption of minerals and results in increased growth and productivity (Brunt and Austin 2005; El-Haroun et al. 2006; Tuan et al. 2013).

Digestibility of crude protein (72%), fat digestibility (75%) and gross energy (69%) were maximum in *Catla* fed on diet containing 2 g/kg of probiotics and minimum excretion of CP in faeces (9.03) was also recorded in the same group II. Likewise, Akrami and Arab Arkadeh (2016) found higher digestibility of nutrients in *Rutilus rutilus* fed on 2 g/kg probiotics included in wheat flour-based diet. Similar to the current results, Mazurkiewicz et al. (2005) and El-Komy and Shehab El-Din (2014) found highest crude protein digestibility in *Cyprinus carpio* and Nile tilapia fed on 1 g/kg level of probiotics supplemented in soybean meal-based diet. Contrary to these findings, Hussain et al. (2018) showed that the fish fed on 3 g/kg level in canola meal-based diet have highest digestibility of crude protein (CP, 13.67%) and crude fat (6%), as compared to fish fed on control diet. Slightly similar to our results recorded by Bagheri et al. (2008), it indicated that fish fingerlings fed on 1×10^7 CFU g^{-1} probiotics included in soybean meal-based diet were found to have better fat digestibility. Contrary to current findings, Silva et al. (2015) reported that in the body of Nile tilapia fed *Bacillus amylolique faciens*-supplemented plant meal-based diet at a rate of 1×10^6 CFU g^{-1}, there was maximum digestibility of crude protein and gross energy. Makled et al. (2019) delineated that feeding diets supplemented with probiotics (5 g/kg) resulted in improved protein efficiency ratio (PER 2.7%) in *Oreochromis niloticus*.

Rainbow trout fed on soybean-based diet supplemented with probiotics i.e. 1.5×10^8 CFU/g showed maximum protein and crude fat digestibility particularly in comparison to fingerlings fed on control diet (Sealey et al. 2009). Yanbo and Zirong (2006) noted that probiotics supplementation

(*Bacillus* species 10^{11} CFU g^{-1}) improves activity of enzymes in the digestive tract of common carp (*Cyprinus carpio*) leading to higher digestibility values. Contrary to these results, Dhanaraj et al. (2010) reported better digestibility of protein (48%) and lipid (18%) in koi carp fingerlings fed on 0.5% probiotics included in soybean-based diet. Another experiment conducted by Mohapatra et al. (2012) showed improved apparent digestibility of crude fats (75%) and proteins (86%) in *Labeo rohita* when fed on probiotics, containing basal diet, *Bacillus subtilis*, *Lactococcus lactis* and *Saccharomyces cerevisiae* (10^{11} CFU kg^{-1}) included in soybean meal-based diet. Tuan et al. (2013) reported that usage of plant meal-based diet supplemented with probiotics resulted in higher nutrient digestion (CP, fat and gross energy) without any negative effect. Whereas, some studies have argued that probiotics supplementation has no positive impacts on overall results, including nutritional value in fish (Telli et al. 2014). The variability in these findings might be due to the efficacy of probiotics depending on the fish species and their defence mechanisms (Son et al. 2009; Merrifield et al. 2010).

Current results showed that the probiotics addition in rapeseed meal-based diet is much advantageous for mineral absorption in Thaila fingerlings fed on diet consisting of 2 g/kg of probiotics. Similar to our results, another study by Riaz et al. (2018) recorded that 2 g/kg is an optimum level for maximum mineral absorption in rohu fed on *Moringa olefera-based* diet. Maximum digestibility of Ca (72%), Na (75%), K (70%) and P (69%) were found at 2 g/kg, whereas few other minerals (Cu, 62% and Mg, 63%) were better absorbed at 4 g/kg level of probiotics. Quite similar results were also found in this study conducted by Hussain et al. (2018) which showed better absorption of various minerals (Ca, 76%; Fe, 62%; P, 76%) occurred at 3 g/kg, whereas few other minerals absorption (Na, 75%; K, 67%; Mg, 62%) occurred at 4 g/kg probiotics-supplemented canola meal-based diet in *Oreochromis niloticus* fingerlings. Similar to present results, Rodrigues et al. (2012) delineated that maximum absorption of mineral contents such as Ca, P and Mg in animals occurred when fed with probiotics (*Bifidobacterium longum*) supplemented in yacon flour. Probiotics inclusion in fish diets favours microbial communities in the intestine that may alter the morphology of intestines and produce certain enzymes resulting in better absorption of nutrients and minerals (Verschuere et al. 2000). The impacts of probiotics have been linked to regulation of GI microbiota and development of beneficial microbes and augmented total digestive enzyme activity and higher specificity in membranes, leading to better nutrient digestion and feed utilization (Watson et al. 2008).

An important factor in aquaculture reflecting the production yield is growth rate, which may be influenced by a number of factors including environmental factors, genetic factors and by quality and quantity of food. Maximum growth performance in terms of weight gain (20%) and weight gain percentage (303%), FCR (1.22) and SGR (1.55) observed in *C. catla* fed on 2 g/kg of probiotics followed by 1 g/kg. Like the above mentioned results, Akrami and Arab Arkadeh (2016) observed improved growth performance with 2 g/kg level in Caspian roach. Similar to our results, another study conducted by Van Doan et al. (2019) reported that Pangasius catfish (*Pangasius bocourti,* Sauvage 1880) fed on 3 g/kg of Eryngii mushroom (*Pleurotus eryngii*) supplemented with *Lactobacillus plantarum* (10^9 CFU ml^{-1}) indicated better growth performance, higher SGR and lower FCR values were recorded. Another 2-month trial was conducted by Dawood et al. (2016) by using *Bacillus subtilis*, *Lactococcus lactis* and *S. cerevisiae* which showed better growth, significant increase in weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio in major carp, *L. rohita* fingerlings feeding on diets containing 10^{11} CFU kg^{-1} feed. Improvement in growth parameters particularly final weight gain (3.26 g) and SGR (2.51) was also observed in *L. delbrueckii* in comparison to fish fed on control diets, and the optimal values were recorded at 1×10^6 CFU/g (Zhang et al. 2019). Giri et al. (2013) recorded that supplementation of probiotics (10^8 and 10^{10} CFU g^{-1}) in *L. rohita* improved weight, SGR and FCR when compared to control diet fed fish. Gupta et al. (2016) observed that supplementation of probiotics microbial strains (*Bacillus coagulans (MTCC 9872), B. licheniformis (MTCC 6824) and Paenibacillus polymyxa MTCC 122*) at level of 10^9 CFU/g in basal palleted SBM diet resulted in better effects on growth (FCR 4.26; SGR 1.76 PER 0.70 and final body weight 1.35 g) of common carp. Maximum SGR was also reported by Van Doan et al. (2019) in Nile tilapia fed on diet having a combination of orange peels with probiotics inclusion level of 10^8 CFU/g. Yih et al. (2019) also noted significant increase in weight gain at 10^7 CFU/g probiotics in tilapia fed on soybean meal-based diet. Another study conducted by Valiallahi et al. (2018) showed that addition of *Lactobacillus plantarum* in soybean meal-based diet at the level of 4.5×10^6 CFU/mg resulted in increased growth performance, better protein efficiency ratio (1.34%), highest SGR (2.61) and lowest FCR (2.35) and were recorded at this level in common carp.

The current results indicate that levels of probiotics from 1 to 3 g/kg induce best growth results. In contradiction to our results, Makled et al. (2019) monitored the highest growth rate at 5 g/kg in *Oreochromis niloticus*, and growth performance was significantly declined at 20 g/kg. Contrary to our results, Bhatnagar and Saluja (2019) observed the best growth results (final weight, 3.21 g; WG, 2.15; SGR, 0.48% BWd^{-1}; lowest FCR, 2.53 and apparent protein digestibility APD, 79%) in *C. catla* fed on probiotics containing *B. coagulans* at optimum level of 3000 CFU g^{-1}, supplemented in 6 g/kg of *Mentha piperita* (peppermint) meal-based diet. Numerous studies indicated that almost all probiotics bacteria improve various growth parameters through release of multiple nutrients, which increase growth; whereas, Ahmadvand et al. (2012) reported that there was no increase in the FCR and SGR results when diets were supplemented with probiotics in rainbow trout. Probiotics included in plant-based meals seem to have no influence on growth performance in fresh water fishes, according to some researchers (Gomez-Gil et al. 2000; Irianto and Austin 2002). Telli et al. (2014) found no substantial differences in growth rates of tilapia when fed on probiotics-supplemented diet and without supplementation. These differences in probiotics rates for better results in various fishes might be due to variable fish species, diet quality and probiotics used in feed and experimental conditions (Bongers and van den Heuvel 2003).

Overall, our results delineated the fact that probiotics improve gut microbiota thus better absorption of minerals and nutrients leads to better growth in *C. catla* fingerlings. The underlying mechanism that leads to better growth is the fact that addition of probiotics has significant effects on digestive enzyme functions causing increase in nutrition of host species through increase in production of digestive enzymes and pre-digestion of anti-nutritional factors present in feed (Verschuere et al. 2000).

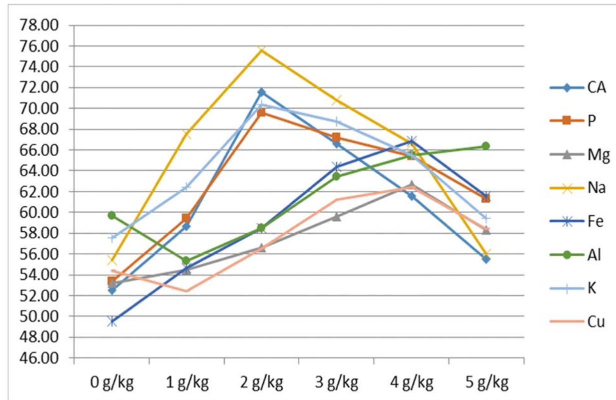

Fig. 2 Graphical presentation of mineral absorption in C. *catla* fingerlings fed probiotics supplemented rapeseed meal based diets

Conclusion

In conclusion, the current study designates the fact that optimum inclusion of 2 g/kg of probiotics to the feed leads to better growth of microflora in intestines, thus supporting absorption of minerals and nutrients resulting in better growth performance. It has been concluded that a diet based on rapeseed meal proves to be valuable in establishing environmentally safe feed for *C. catla* when supplemented with probiotics, thus avoiding pollution. It has also been estimated that a diet based on 2 g/kg probiotics level is appropriate and plays an important role in enhancing growth performance, digestibility of nutrients and mineral absorption in fingerlings of *C. catla* (Figs. 1, 2, 3).

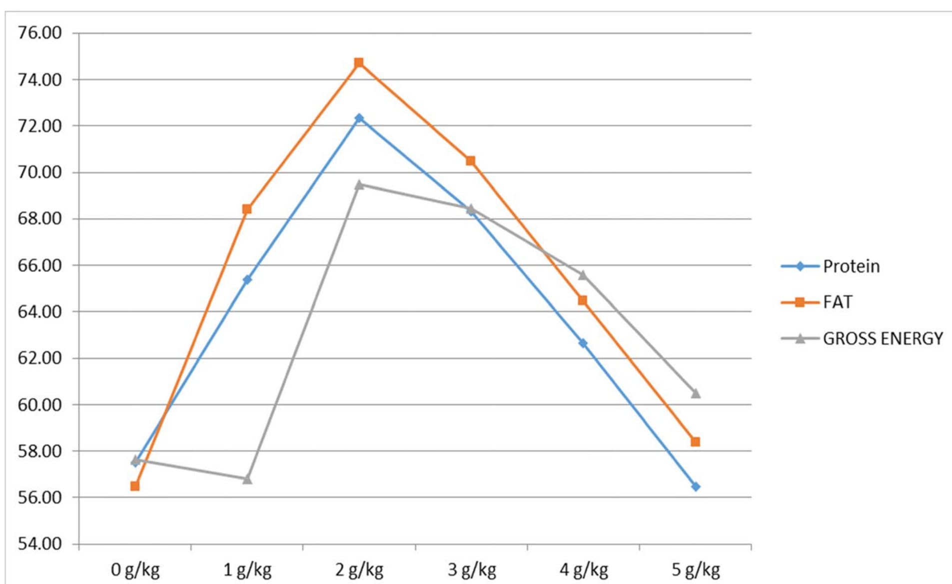

Fig. 1 Graphical presentation of nutrient digestibility in C. *catla* fingerlings fed probiotics supplemented in rapeseed meal based diets

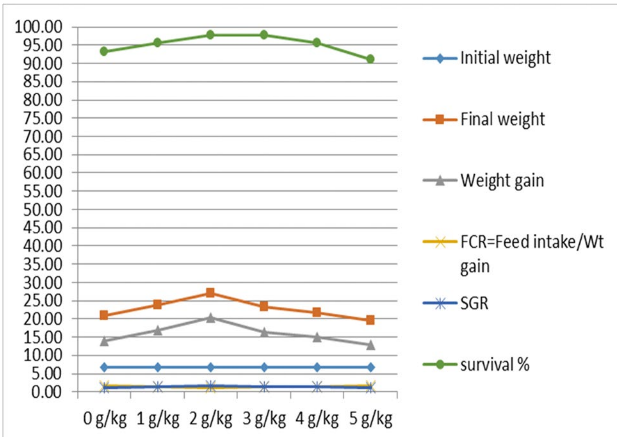

Fig. 3 Graphical presentation of growth in C. *catla* fingerlings fed probiotic in rapeseed meal based diets

Author contribution Muhammad Mudassar Shahzad planned and supervised the research and provided the research materials. Syed Makhdoom Hussain helped in the statistical analysis. Majid Hussain proofread the manuscript. Naveed Ahmad prepared the manuscript. Laraib Tahir conducted the trial and collected the data while Kalsoom Akhtar helped her.

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Data availability Supplementary material is available in our another article in the Pakistan Journal of Zoology https://dx.doi.org/10.17582/journal.pjz/20211011061010.

Declarations

Ethical approval The manuscript is not submitted to any other journal for publication and this is original work. Results were presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. No data, text, or theories by others are presented as if they were the author's own ("plagiarism").

Consent to participate N/A

Consent for publication All the authors gave consent for the publication of this journal.

Conflict of interest The authors declare no competing interests.

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