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EFFECTIVENESS OF PROBIOTICS SUPPLEMENTED LINSEED MEAL BASED DIETS ON OVERALL PERFORMANCE OF *LABEO ROHITA*

M. M. Shahzad[1], K. Akhter[1], S. M. Hussain[2], Z. Hussain[1], M. Y. Zahoor[3], N. Ahmad[2], F. Khalid[1] and M. T. Rafique[1]

[1] Department of Zoology, Division of Science and Technology, University of Education, Lahore, Pakistan
[2] Fish nutrition Lab, Department of Zoology, Government Collage University Faisalabad.
[3] Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
Corresponding author's email: drmudassarshahzad@gmail.com

ABSTRACT

Probiotics have positive effects on the host by inhibiting pathogenic microorganisms, improving feed intake, contributing enzymatically to digestion, secreting growth-promoting factors and improving the quality of pond water. Seventy days research trial was performed to check the efficacy of prob-added linseed meal based (LMB) diet on overall performance including growth, mineral absorption and digestibility of nutrients in *Labeo rohita* fingerlings. By using various concentrations of probiotics (0, 1, 2, 3, 4, 5 g/kg) six test diets were prepared. All six groups were fed twice a day, their faeces samples were collected on daily basis and stored for chemical analysis. Results of current research revealed that probiotics addition showed significant improvement in fish. Highest weight gain (20g), weight gain% (279%), SGR (1.48) and best FCR (1.21) was found in fish fed 2 g/kg level of prob-added LMB diet. Similarly, highest nutrient digestibility (CP; 71% and Fat; 75%) and mineral absorption (P; 72%, K; 75% and Na; 76) were also analyzed in the juveniles that fed with the above said test diet. Whereas maximum digestibility of GE (74%) and minerals absorption (Ca; 73%, Al; 69%) was observed maximum at test diet IV that was supplemented with 3 g/kg LMB diet. On the basis of these results, it was concluded that 2g/kg level of probiotics inclusion was optimum for the formulation of ecofriendly and economically affordable fish diet by using LMB diet.

Keywords: Probiotics, Growth performance, Digestive enzymes, Nutrient digestibility, Minerals

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INTRODUCTION

World population is increasing day by day and in 2050 the world population is expected to grow up to about 9 billion. There is an increasing demand of food with continuously increasing world population (Godfray *et al.,* 2010). Aquaculture plays a significant role in supplying the feed to the ever increasing world population with safe livelihood opportunities and food security (Faggio *et al.,* 2014; Dawood *et al.,* 2016). Aquaculture is found as one of the most developing industry for supplying of food to the world's population in term of animal protein (De *et al.,* 2014). However, aquaculture faces various challenges; such as preparation of suitable, low cost feed and feeding systems, fighting against diseases, improving domesticating brood stock and maintaining the water quality. The feed must be nutritiously balanced with adequate amount of protein, carbohydrates and minerals to achieve the targeted higher growth in aquaculture (Ayyappan and Jena 2003). In the subcontinent, the most cultivable and preferable fish is rohu (*L. rohita*). Due to its delicious taste, *L. rohita* has a high market demand and profit values (Khan *et al.,* 2004).

Owing to the high digestibility and deliciousness, fishmeal (FM) is an excellent source of protein in feed and contains good mineral concentrations, EAA as well as EFA (Olsen and Hasan 2012). But FM supply becomes significantly low due to their huge demand and high prices in the market. So due to its finite supply and increasing costs, research centers and aquaculture feed industry has conducted different experiments to decrease dependence of fish feed on FM (Hussain *et al.,* 2018). With rapid increase in the development of aquatic animal's culture there is a global need for cheaper and nutritionally healthy feed ingredients for commercial diets to satisfy this increasing need for the aquaculture industry (FAO 2016). Through reducing feed price, the use of locally available inexpensive and nutritionally appropriate non-conventional feed in the fish diet will undoubtedly have a significant impact on the carp culture (Kumar *et al.,* 2006).

Different plants by products, such as rapeseed, soybean, sunflower, corn gluten etc. are used as alternative sources of protein (Shahzad *et al.,* 2021). Linseed that is known as flaxseed is the plant by which linseed meal can be prepared after oil extraction. Flaxseed is an important functional feed ingredient because it provides omega-3, digestible proteins and lignans. Flaxseed (low cost as well as easily and consistently available) is an essential source of high quality protein and soluble fiber (Oomah 2001; Pengilly, 2003). An alternative approach to reduce adverse factors of PPs is the inclusion of probiotics that can improve growth performance of fish and nutrients intake. Diets supplementation with probiotics increases the growth performance, improves disease resistance, immunological parameters, body composition and microbial balance of fish (Faramarzi *et al.,* 2011; Akhter *et al.,* 2015). Fingerlings of fish do not have a fully mature digestive system and lack a variety of microbiota (Ghosh *et al.,* 2004). Survival and growth rates of fish could be improved by addition of probiotics that will enhance digestive enzymes activity (Liu *et al.,* 2010) and also increase fish appetite (Nuenen *et al.,* 2005). Probiotics are considered as a new approach for promoting growth and improving the water quality in aquaculture, enhance stress tolerance in fish, efficiency of fish species, improve the use of nutrients and growth performance of fish by producing digestive enzymes, vitamins and short-chain fatty acids (Mohapatra *et al.,* 2012; Wang *et al.,* 2019). Current research was designed to check the impacts of prob-added LMB diet especially in term of improving performance of growth, digestibility of nutrients and accessibility of minerals to *L. rohita* fingerlings.

MATERIALS AND METHODS

Experimental conditions: Experiment was conducted in fish nutrition lab, Department of Zoology, Division of Science and Technology, University of Education Township campus, Lahore. Fingerlings of similar length and average weight were stocked in V-shaped water tanks with a storage capacity of approximately 70L. Before the starting of trial period; fish were acclimatized with the laboratory conditions for fifteen days. During this time span, the fingerlings were fed with basal diet two times a day (Allan and Rowland, 1992).

Experimental design: Linseed meal was used as a major ingredient for the preparation of six experimental (control and test) diets with different probiotics concentrations at the rate of 0, 1, 2, 3, 4 and 5gkg^{-1} i.e. 0, 0.1, 0.2, 0.3, 0.4 and 0.5%. Fish were distributed into six groups and each group into three replicates. One group was nourished with five with prob-added LMB test diets and one without probiotics (control). V-shaped triplicate tanks were used for each group throughout the trial containing 15 fingerlings each. Fish were fed @4% of live body weight. Total duration (70 days) of experimental trial was conducted following a completely randomized design (CRD). *L. rohita* juveniles fed at control diet were compared with fish fed on test diets to assess the nutrients digestibility, growth and mineral absorption of *L. rohita* fingerlings.

Formation of feed pellets: Linseed meal and all other ingredients were purchased from commercial market and grounded into fine powder to pass through mesh sieve of 0.3 mm. As an indigestible marker, chromic oxide was used in all diets at a rate of 1%. Feed was prepared according to standard methods by addition of specific concentrations (0, 1, 2, 3, 4 and 5 gkg^{-1}) of probiotics into six groups and were dried in air for a short period of time and stored in air tight jars at 4°C.

Collection of samples: Rohu fingerlings were fed with their respective diets two times-a-day and after two hour of feeding, the remaining diet was collected by the help of collection tube for the calculation of FCR. Samples of feces were also taken from tanks by opening valves located at the base of tanks and were stored at room temperature for analysis.

Study of growth indices: Fingerlings (15) with average mass (7g) and length (12cm) were stocked in each tank. Throughout the whole experimental period, fish from each tank were weighed on fortnightly basis for the evaluation of growth performance. Growth parameters like SGR, FCR and WG %, of *L. rohita* fingerlings were calculated by using following formulas (NRC, 1993).

$$WG\% = \frac{(FW - IW)}{IW} \times 100 \qquad (1)$$

$$FCR = \frac{\text{Total dry feed intake (g).}}{\text{Wet WG. (g)}} \qquad (2)$$

$$SGR\% = \frac{(\ln. FW \text{ of fish} - \ln. IW \text{ of fish})}{\text{Trial day}} \times 100 \qquad (3)$$

Nutrient digestibility: After 70-days of feeding trial, homogenized samples of feed and feces were oven dried at about 105ºC for 12 hours for analysis of moisture content of feces and diet. Micro Kjeldahl Apparatus was used to evaluate the contents of crude protein (CP) while crude fat was analyzed by using the Soxhlet Apparatus by petroleum ether-extraction-methods. Crude fiber and ash contents were calculated standard methods (AOAC 1995). To evaluate the gross sample energy, Oxygen bomb calorimeter was used. Total carbohydrates contents were measured by using the following formula.

$$\text{Total carbohydrates (\%)} = 100 - (EE\,\% + CP\,\% + Ash\,\% + CF\,\%) \qquad (4)$$

Estimation of minerals: The mineral contents (Na, Ca, Al, K, Zn, P, Mg, Cu and Fe) were determined by using the Atomic Absorption Spectrophotometer after appropriate dilution according to AOAC (1995). By using flame photometer, the sodium and potassium contents were analyzed. After oxidation with molybdate reagent by using spectrophotometer, chromic oxide contents were estimated at an absorption rate of about 370 nm in diets and feces (Divakaran *et al.,* 2002). UV/VIS spectro-photometer was used to analyzed the phosphorous concentration at an absorption rate of 720 nm of experimental diets and feces. The standard formula was used to calculate the ADC% of nutrient digestibility and mineral absorption of test diets (NRC, 1993).

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

Statistical analysis: Data on ADC% of mineral intake (P, K, Ca, Al, Fe, Na, Mg and Cu) and nutrients (CP, Fat and GE), survival as well as growth parameters (weight gain percentage, SGR and FCR) were analyzed by one-way ANOVA. Tukey's Honestly Significant Difference Test was used to compare the differences among treatments and considered significant at P<0.05. For statistical analysis of samples, CoStat Computer Package was used.

RESULTS

Results of growth showed that prob-added diets enhanced growth indices of juveniles in contrast to control one. It was observed that all the parameters of growth were improved maximum at test diet III (2 g/kg). Decrease in growth of fish was observed in test diet VI supplied with 5 g/kg level of probiotics and control diet. Highest weight gain (20g), SGR (1.48) weight gain% (279%) and survival% (98%) were observed in the fingerlings fed on 2 g/kg prob-added diet followed by weight gain (17.3g), SGR (1.37), weight gain % (245%) and survival (97%) when fish were fed at 3 g/kg level of probiotics and these values were significantly different from each other (Table 2). Lowest weight gain (13 g), SGR (1.13), WG% (176%) and survival % (88%) were found in fingerlings fed with diet VI (5 g/kg). Fish fed on 2 g/kg level of probiotic supplemented diet showed best FCR (1.21) value accompanied by (1.29) fish fed a diet that was supplemented with 3 g/kg level of probiotics. Poor FCR (1.76) was recorded in fingerlings that were fed at 5 g/kg level of probiotics followed by (1.59) when were fed at test diet V (4 g/kg). The less FCR had greatest transformation of feed into fish flesh and lowest discharge through feces. It was indicated from the results that 2 g/kg level of probiotics in feed of rohu is the optimum level for highest fish growth as shown in figure 1. It was also noted that further increase in concentration of probiotics couldn't enhance the growth parameters of fish.

Nutrients in feces released by rohu (when fed test diets prepared by Linseed meal) and digestibility of nutrients (CP, Fat, GE) are shown in Table 3. From analysis it was observed that all respective diets comprise on equivalent quantity of nutrients i.e. CP, CF and GE (Table 1) but different in feces. Findings indicate that when *L. rohita* were fed at test-diet III added with 2 g/kg probiotics level followed by fish fed at 3 g/kg level, there was less nutrient release in feces. Current findings showed that highest ADCs% of nutrient (CP; 71% and Fat; 75%) was observed at 2g/kg level followed by (CP; 70% and Fat; 71%) fish fed with 3 g/kg and these values were significantly different from each other. Highest ADCs% of gross energy (74%) was observed at test diet IV with 3 g/kg level of probiotics and second highest value (72%) was observed at test diet III. These values were different from control and other test diets supplemented with different concentrations of probiotics. However minimum digestibility of nutrients (CP; 55%, GE; 53%) were observed in fingerlings fed with control diet (0 g/kg) and lowest digestibility of fat (54%) was observed at test diet VI (5 g/kg) with highest concentration of probiotics. From these findings it was noted that digestibility of nutrient was increases from 1 g/kg and reaches maximum at 2 g/kg (CP, Fat) and 3 g/kg (GE) and decline was observed on further higher concentrations (figure 2). Further on it was concluded that 2 and 3 g/kg prob-added LMB diet was most suitable for the fingerlings of rohu.

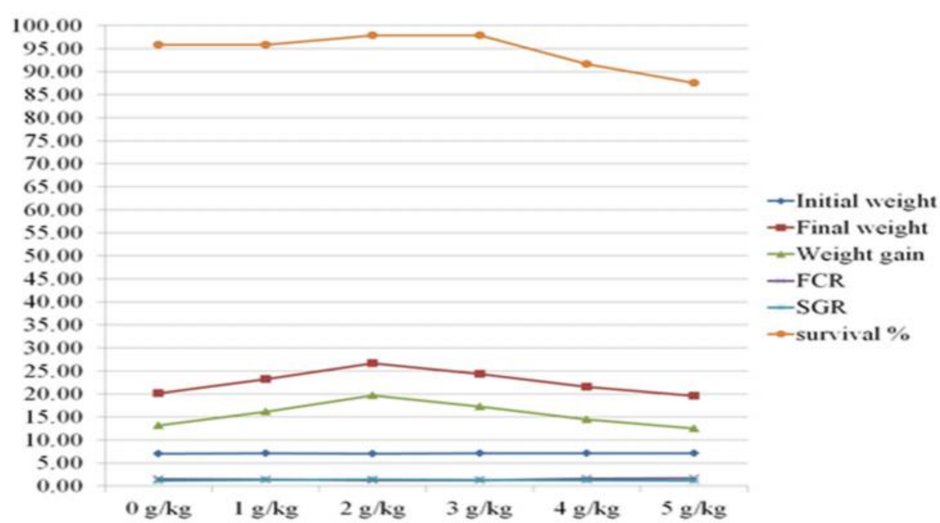

Figure 1: Growth of rohu fingerlings fed graded levels of probiotics added diet

After analysis of diets it was observed that there was a significantly similar amount of all the minerals (P, Ca, Na, Mg, Fe, Al, Zn and K) in all test diets prepared by using LMB diets with probiotics supplementation as shown in table 4. Current findings revealed that absorption of minerals like P, Ca, Na, Mg, Al and K in all test diets were increased by using probiotics levels upto 3 g/kg level based diet. Analysis of feces released by rohu showed statistical differences in quantity of minerals in fish feeding on control and test diets as shown in table 5. Results suggested that significant quantities of minerals were excreted into water by feces when rohu was fed on control diet.

Table 6 indicated that absorption of minerals was minimum at control diet (0g/kg) and increasing trend was recorded from 1g/kg to 3 g/kg level of prob-added LMB diet. On the other hand, it was noted that further increased in probiotics level decreased the mineral absorption. Highest mineral absorption of P (72%), K (75%), and Na (76%) was observed at 2 g/kg level of probiotics followed by (P; 71%, K; 70% and Na; 72%) when fed on 3 g/kg level of probiotics and these values were significantly different from each other. Highest absorption of Ca (74%) and Al (69%) was observed at test diet IV (3 g/kg) and followed by (Ca; 73%) test diet III with 2 g/kg level of probiotics. Maximum absorption of Fe (72%) was observed at 4 g/kg level of probiotics. Other minerals like Mg (70%) and Cu (69%) showed their best absorption at 5 g/kg and second highest absorption value of Mg (68%) and Cu (65%) was observed at test diet V with 4 g/kg level of probiotics. Lowest absorption of P (54%), Ca (57%), Fe (56%) and Cu (53%) were observed at test diet I (control diet; 0 g/kg) while Na (60%), K (61%) and Al (59%) mineral showed lowest absorption at test diet VI (5 g/kg) and lowest absorption of Mg(57%) was noted at test diet II (1 g /kg) as shown in figure 3.

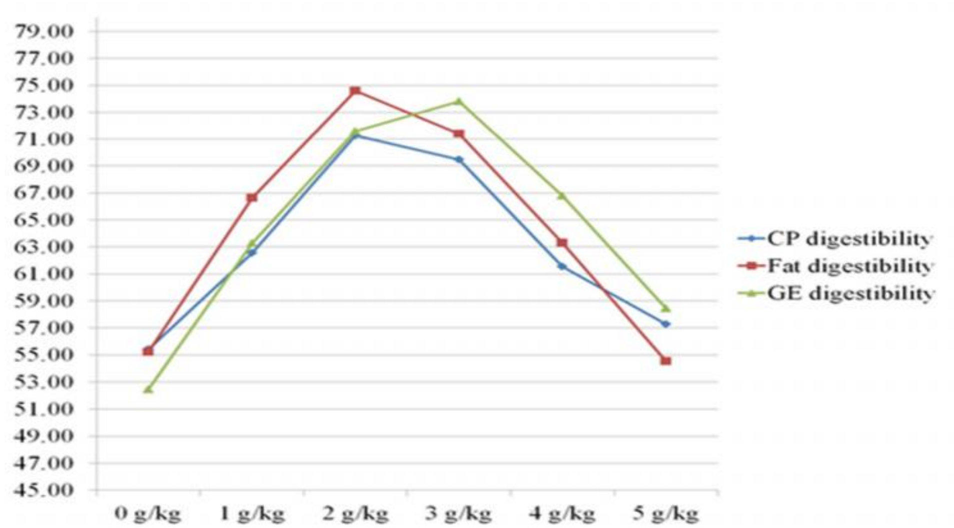

Figure 2: Graphical presentation of nutrient digestibility in *L. rohita*

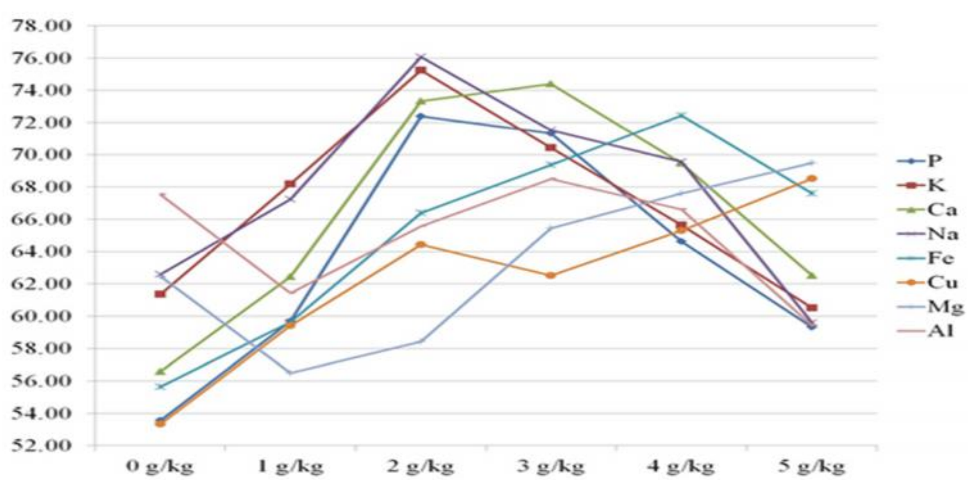

Figure 3: Graphical presentation of ADC% composition of mineral absorption for Rohu fingerlings fed prob-added LMB diets

Table 1. Composition of test diets and chemical analysis of ingredients (dry matter).

Ingredients	Test diet composition	Ingredients and their composition (Chemical)						
		Dry-matter(%)	GE(Kcal/g)	CP (%)	EE (%)	Crude fiber (%)	Ash (%)	Carbohydrates
Linseed Meal	35	92.41	3.86	33.78	6.83	2.76	6.51	50.12
Fish Meal	13	91.93	3.92	45.13	8.46	1.7	21.53	23.18
Wheat Flour*	13	91.81	2.8	9.69	2.27	2.68	3.49	81.87
Rice Polish	12	93.59	4.08	13.59	10.09	3.9	6.83	65.59
Sunflower	14	92.03	4.11	32.68	5.74	2.54	2.43	56.61
Fish Oil	9							
Vitamin Premix**	1							
Chromic Oxide	1							
Mineral Premix***	1							
Ascorbic Acid	1							
Probiotics****				0-5g/kg (0.0-0.5%)				

* Probiotics were incorporated at cost of wheat-flour

** Vit. E (300,00 IU), Vit. D3 (300,000,0 IU), Vit. A (150,000,00 IU), Vit. B1 (3000 mg), Vit. B2 (7000mg), Vit. B6 (4000mg), Vit. B12 (40mg), Vit. C 15000mg), Vit. K3 (8000mg), Nicotinic Acid (60000mg), Calcium Panthenate (12000mg), Folic Acid (1500mg).

*** P (135g), Na(45g), Fe(1000 mg), Ca(155g), Mg(55g), Cu(600mg) **** *Lactobacillus acidophilus Bifidobacterium lactis, L. bulgaricus B. bifidum. B. bifidum, L. rhamnosus, Sreptococcus thermophiles,*

Chemical composition of control and test diets based on LMB diet

Diets	Prob levels	Protein	Fat	Gross energy
TD-I (Control)	0	31.8±31.80	6.83±0.20	3.82±0.20
TD-II	1	31.82±0.12	6.81±0.27	3.82±0.20
TD-III	2	31.80±0.21	6.82±0.17	3.83±0.18
TD-IV	3	31.80±0.21	6.84±0.14	3.81±0.22
TD-V	4	31.83±0.23	6.82±0.23	3.81±0.18
TD-VI	5	31.82±0.20	6.81±0.23	3.84±0.22

All the values are means of three replicates with fifteen fingerlings in each.

Table 2. Growth indices of rohu (fingerlings) fed prob-added LMB diets

Diets	Prob levels	IW	FW	WG	WG%	Survival %	Feed intake	FCR	SGR
TD-I (Control)	0	7.00±0.13	20.16±0.67[d]	13.16±0.60[d]	187.85±7.35[d]	95.83±3.61[ab]	0.23±0.0	1.55±0.0[b]	1.17±0.03[d]
TD-II	1	7.09±0.24	23.19±0.75[bc]	16.11±0.51[bc]	227.30±0.83[bc]	95.83±3.61[ab]	0.25±0.02	1.41±0.08[c]	1.32±0.00[bc]
TD-III	2	7.05±0.17	26.70±0.79[a]	19.64±0.69[a]	278.52±8.51[a]	97.92±0.02[a]	0.25±0.02	1.21±0.0[d]	1.48±0.02[a]
TD-IV	3	7.07±0.16	24.36±0.81[b]	17.29±0.97[b]	244.88±18.98[ab]	97.92±3.61[a]	0.25±0.02	1.29±0.0[d]	1.37±0.06[ab]
TD-V	4	7.07±0.24	21.57±0.83[cd]	14.50±1.07[cd]	205.45±22.08[cd]	91.67±3.61[ab]	0.26±0.02	1.59±0.0[b]	1.24±0.08[cd]
TD-VI	5	7.09±0.20	19.57±0.83[d]	12.48±0.63[d]	175.95±4.24[d]	87.50±0.00[b]	0.24±0.01	1.76±0.04[a]	1.13±0.02[d]

IW : Initial Weight, FW : Final Weight, FI : Feed Intake

[a-f]Means within column having unlike superscripts (a-f) are quietly different at $P<0.05$.

All the values are means of 3 replicate with fifteen fingerlings in each.

Table 3. Analyzed nutrients composition in feces and apparent digestibility coefficient percentage of CP, fat and gross energy in *L. rohita*

Diet	Prob levels	Fat in feces	Fat Digestibility	CP in feces	CP Digestibility	GE in feces	GE Digestibility
TD-I (Control diet)	0	3.22±0.09[a]	55.21±0.86[e]	14.94±0.18[a]	55.4±0.72[c]	1.92±0.11[a]	52.45±0.85[e]
TD-II	1	2.40±0.11[b]	66.6±0.86[c]	12.56±0.38[c]	62.57±0.64[b]	1.48±0.12[bc]	63.28±0.96[c]
TD-III	2	1.8±0.07[c]	74.58±0.98[a]	9.60±0.22[d]	71.27±0.85[a]	1.14±0.07[de]	71.5±0.93[a]
TD-IV	3	2.07±0.09[c]	71.40±0.83[b]	10.27±0.29[d]	69.48±0.77[a]	1.06±0.11[e]	73.79±0.96[a]
TD-V	4	2.62±0.14[b]	63.29±0.67[d]	12.82±0.13[c]	61.53±0.93[b]	1.32±0.07[cd]	66.79±0.95[b]
TD-VII	5	3.22±0.11[c]	54.52±0.74[e]	14.15±0.37[b]	57.26±0.95[c]	1.66±0.07[b]	58.46±0.81[d]

CP = Crude protein, GE = Gross energy

[a-f]Means within column having dissimilar superscripts (a-f) are quietly dissimilar at *P<0.05*.

All the values are means of 3 replicate with fifteen fingerlings in each.

Table 4. Percentage composition of minerals in control and tests diet based on linseed meal.

Mineral	Control Diet (Test Diet I)	Test Diet II	Test Diet III	Test Diet IV	Test Diet V	Test Diet VI
	Probiotic levels (g/kg)					
	0	1	2	3	4	5
Na	0.02±0.01	0.03±0.01	0.02±0.00	0.02±0.01	0.02±0.01	0.0±20.00
P	2.11 ±0.10	2.10±0.09	2.10±0.12	2.09±0.13	2.09±0.10	2.08±0.10
Fe	0.06±0.01	0.06±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.05±0.01
K	1.39±0.11	1.37±0.11	1.38±0.14	1.39±0.09	1.36±0.12	1.38±0.11
Ca	0.95±0.06	0.96±0.08	0.95±0.05	0.96±0.07	0.94±0.08	0.96±0.06
Cu	0.0064±0.00065	0.0063±0.00061	0.0064±0.00065	0.0064±0.00072	0.0065±0.00060	0.0065±0.00056
Al	0.000580±0.000040	0.000590±0.000050	0.000570±0.000036	0.000593±0.000050	0.000580±0.000046	0.000593±0.000067
Mg	0.00970±0.00098	0.00953±0.00090	0.00940±0.00050	0.00967±0.00108	0.0095±0.00080	0.00950±0.00072

All the values are means of 3 replicate with fifteen fingerlings in each

Table 5. Percentage minerals composition in fish feces fed prob-added LMB diets

Minerals	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
	Probiotic level (g/ kg)					
	0	1	2	3	4	5
P	1.03±0.08[a]	0.89±0.02[ab]	0.61±0.05[d]	0.64±0.08[cd]	0.77±0.05[bc]	0.88±0.05[ab]
Na	0.009±0.003[a]	0.009±0.002[a]	0.007±0.002[a]	0.008±0.003[a]	0.010±0.003[a]	0.005±0.001[a]
K	0.57±0.05[a]	0.46±0.05[abc]	0.36±0.03[c]	0.43±0.03[bc]	0.49±0.03[ab]	0.57±0.05[a]
Ca	0.44±0.04[a]	0.38±0.04[ab]	0.27±0.03[c]	0.26±0.03[c]	0.30±0.03[bc]	0.38±0.02[ab]
Fe	0.026±0.004[a]	0.024±0.003[ab]	0.019±0.003[abc]	0.018±0.002[bc]	0.016±0.002[c]	0.018±0.002[bc]
Al	0.00020±0.00001[b]	0.00024±0.00001[ab]	0.00021±0.00002[b]	0.00020±0.00001[b]	0.00020±0.00020[b]	0.00025±0.00030[a]
Mg	0.0038±0.0003[abc]	0.0044±0.0004[a]	0.0041±0.0001[ab]	0.0035±0.0004[abc]	0.0033±0.0003[bc]	0.0030±0.0003[c]
Cu	0.0031±0.0003[a]	0.0027±0.0003[ab]	0.0024±0.0003[b]	0.0025±0.0003[ab]	0.0023±0.0003[b]	0.0021±0.0002[b]

[a-f]Means in a row having dissimilar superscripts (a-f) are quietly dissimilar

All the values are means of 3 replicate with fifteen fingerlings in each

Table 6. Analyzed composition of minerals absorption in *L. rohita* fed prob-added LMB diets.

Minerals	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
	Probiotics level (g/kg)					
	0	1	2	3	4	5
P	53.56±0.90^{d}	59.69±0.96^{c}	72.40±0.62^{a}	71.32±0.98^{a}	64.62±0.92^{b}	59.32±0.99^{c}
Na	62.60±0.98^{d}	67.23±0.99^{c}	76.06±1.48^{a}	71.52±0.83^{b}	69.55±0.92^{bc}	59.59±0.81^{e}
K	61.35±0.73^{d}	68.17±0.99^{b}	75.24±0.86^{a}	70.42±0.98^{b}	65.64±0.84^{c}	60.51±0.75^{d}
Ca	56.58±0.74^{d}	62.45±0.93^{c}	73.33±0.95^{a}	74.40±0.93^{a}	69.46±0.96^{b}	62.52±0.80^{c}
Fe	55.65±0.66^{e}	59.63±0.80^{d}	66.38±0.88^{c}	69.36±0.89^{b}	72.43±0.98^{a}	67.61±0.93^{bc}
Al	67.53±0.96^{ab}	61.42±0.79^{c}	65.56±0.82^{b}	68.49±0.74^{a}	66.59±0.86^{ab}	59.43±0.84^{c}
Mg	62.48±0.99^{c}	56.50±0.83^{d}	58.42±0.64^{d}	65.43±0.91^{b}	67.59±0.63^{ab}	69.49±0.84^{a}
Cu	53.34±0.94^{e}	59.41±0.80^{d}	64.44±0.86^{bc}	62.52±0.87^{c}	65.29±0.97^{b}	68.52±0.64^{a}

$^{a-f}$Means in a rows having dissimilar superscripts (a-f) are dissimilar
All the values are means of 3 replicate with fifteen fingerlings in each

So, current results revealed that addition of probiotics in LMB diet at 2 g/kg level is helpful in growth improvement, maximum absorption of minerals and highest nutrient digestibility. It was also concluded that less excretion of minerals and nutrients in water minimize the contamination of water and cost of feed that ultimately beneficial for both fish and farmer.

DISCUSSION

The use of probiotics in aquaculture is becoming a new trend because of its function in enhancing immunity, defending against pathogens, supporting digestion, improving the consistency of the water and thus enhancing growth and reproduction (Gomez-Gil *et al.,* 2000; Austin, 2002; Banerjee and Ray, 2017). Our research work was carried-out to examine the role of commercial prob-added LMB diet on growth performance of *L. rohita.* Maximum WG, SGR, WG% and best FCR were observed in *L. rohita* fed on diet III containing 2g/kg (1g= 2× 10^{9} CFU/g) probiotics. Nearly similar findings to our results, maximum specific growth rate (SGR) and best FCR were observed in rohu and rainbow trout fingerlings fed on 1.5 g/kg level of prob-added plant meal based diets (Krishnaveni *et al.,* 2013; Bhujel *et al.,* 2019). Similarly in two other studies, results revealed that maximum improvement in the growth of tilapia was observed at 3 g/kg level of prob-added diet (Standen *et al.,* 2016; Ramos *et al.,* 2017). Similar to this study, Ghosh *et al*. (2003) revealed that *B. circulans* probiotics supplementation in diets of rohu had the highest growth performance at about 2×10^{5} cells per 100 g of feed. Sahandi *et al.* (2019) observed highest weight gain and lowest feed conversion ratio (P<0.05) in *L. rohita* fingerlings that were fed on diet containing 1×10^{7} CFU/g bacterial concentration and these findings were totally different from our results. Ahmadifar *et al.* (2019) showed that 1×10^{8} CFU/g and 1×10^{9} CFU/g concentrations of *P. pentosaceus* used as a beneficial probiotics were found to enhance the growth performance of common carp and these findings are significantly different form our outcomes. Similarly, Adineh *et al*. (2013) observed significantly better growth at 3×10^{5} CFU/g. Whereas, Kumar *et al*. (2016) investigate that 4g/kg addition of proteus probiotics gave the highest weight gain (4.9 g), SGR (0.521%) and improved FCR. In contradiction to outcomes, Makled *et al*. (2019) monitored highest growth rate at 5 g/kg in *Oreochromis niloticus* and growth performance was significantly declined at 20 g/kg of prob-added plant based diet. After a feeding period of 50 days, the results revealed that all growth parameters including growth rate, final weight (FW), percent weight gain, protein efficiency ratio, feed conversion ratio and productive protein values (PPV) were statistically (P<0.05) improved with bacterial inclusion of up to 5 g/kg. Hassani *et al.* (2020) conducted an experiment and give significantly different results. Their results indicated that 300 mg/kg probiotics used in the feed of *Acipenser baerii* fingerlings to increase growth performance and there was a considerable increase in the weight-gain% and SGR in fish fed prob-added diet compared with the control diet. Whereas, in contrary to these current outcomes, Telli *et al.* (2014) found no impacts of probiotics (5 × 10^{6} CFU/g) supplemented diets on growth performance in Nile tilapia fingerlings. Literature review showed that probiotics are very vital in diets prepared using plant meal for the proper digestion and growth of fish but on different levels due to different circumstances in different fish species. These variations in results are might be due to probiotics type, feed synthesis methods, feed composition, drying and feed storing methods and fish species (Yanbo and Zirong, 2006).

Probiotics release enzymes that cause lysis of carbohydrates, lipids and proteins into smaller units, thus enhancing the absorption of nutrients (Liu *et al*., 2009). Results of this research indicated that probiotics added diet cause significant increase in nutrient digestibility.

Maximum digestibility of nutrients (CP; 71%, CF; 74%) were noted at 2 g/kg of probiotics concentration in LMB diet whereas maximum gross energy (73%) was observed at test diet IV with 3 g/kg level of probiotics. Slightly similar to our results, maximum protein digestibility in tilapia fingerlings fed at 1 g/kg level of prob-added soya-meal (El-Komy *et al*., 2014). Hussain *et al*. (2018) found, fish fed on 3 g/kg probiotics level have maximum protein digestibility in contrast to control. Ghosh *et al*. (2003) reported that probiotics (1.5 x 10^5/100g) concentration should higher digestibility of protein in fish. According to them, lipid digestibility was significantly decreased at higher inclusion rates of probiotics in the rohu fingerlings. Silva *et al*. (2015) reported different outcomes they noted that Nile Tilapia fed on linseed meal supplemented with probiotics at a rate of 1×10^6 CFU g^{-1} gave higher gross energy and crude protein digestibility. Sahandi *et al*. (2019) observed highest nutrient utilization at 1×10^7 $CFUg^{-1}$ in rohu fingerlings. Mohapatra *et al*. (2012) conducted a research and finding revealed that best digestibility of CP was observed at 1×10^{11} CFU /kg concentration of probiotics. They found that there was no significant difference in apparent lipid digestibility in fish fed on probiotics added diet and these findings were different from our findings. Gosh *et al*. (2004) discovered that highest protein digestibility (35.9%) was found in replicate of fish fed on prob-added diet at 0.5g/100g. On the other hand, several studies have argued that probiotics supplementation has no significant impact on overall efficiency including digestibility of nutrient in fish (Grisdale-Helland *et al*., 2008; Mohapatra *et al.,* 2012). Variations in these findings may have been attributed to the efficacy of probiotics depending on the fish species and their defensive mechanism.

Minerals play important role in enzyme functioning including digestion, exchange of gases, acid / base balance and osmoregulation (Ng and Romano, 2013). Results of this research indicated that mineral absorption of K (75%) and P (72%) were observed maximum at 2 g/kg while Ca (74%) and Al (68%) were highest at 3 g/kg supplementation level of probiotics. Riaz *et al*. (2018) demonstrated that probiotics supplementation at 2 g/kg level is the best level for improving overall performance of *L. rohita* fingerlings and these findings were significantly similar to our results. Lowest minerals excretion was noted at 3 g/kg that confirms this level was best for highest fish performance (Hussain *et al*., 2018). Rodrigues *et al*. (2012) demonstrated higher absorption of mineral content in fish fed on Yacon flour supplemented with probiotics. Probiotics tend to enhance the development of beneficial microbial populations, in the gastrointestinal tract, which can modify the morphology of the gut and secrete some enzymes and inhibitory compounds, resulting in increased digestibility of nutrients and minerals absorption (Verschuere *et al*., 2000). Various researchers showed that the probiotics use in the diet enhances the health of larval and juvenile fish, growth efficiency and body structure, but mechanism of probiotics working on different fishes may vary from freshwater fish to marine ecosystem (Kesarcodi- Watson *et al*., 2008). Effects of probiotics were observed on minerals absorption in fish fed with prob-added plant meal based diet (Bongers and Heuvel 2003). All of the differences in results may be due-to different species/types of probiotics, fish and diet quality as well as laboratory environments.

It was concluded from the discussion that prob-added LMB diet was beneficial for the overall performances of *L. rohita* fingerlings. In accordance with current findings, it was concluded that 2g/kg level of probiotics inclusion was optimum for the formulation of ecofriendly and economically affordable fish diet by using LMB diet.

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