International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 105-116, 2018

INNSPUB

RESEARCH PAPER OPEN ACCESS

Mineral digestibility of *Labeo rohita* fingerlings fed on *Moringa oleifera* seed meal based diets supplemented with probiotics

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Key words: *L. rohita*, MOSM, Probiotics, Mineral digestibility

http://dx.doi.org/10.12692/ijb/13.1.105-116 Article published on July 15, 2018

Abstract

Probiotics are becoming an integral part of the aquaculture practices due to their nutritional and other beneficial activities in fishes. This research work was conducted to evaluate the effect of probiotics supplementation on mineral digestibility of *Labeo rohita* fingerlings fed *Moringa oleifera* seed meal (MOSM) based diets supplemented with graded levels (0 gKg^{-1}, 0.5 gKg^{-1}, 1 gKg^{-1}, 1.5 gKg^{-1}, 2 gKg^{-1}, 2.5 gKg^{-1} and 3 gKg^{-1}) of probiotics in fish feed to formulate one control and six test diets. Three replicate groups of 15 fingerlings were fed at rate of 4% of live wet weight on their prescribed diets and feces were collected twice daily. Chromic oxide was added in the feed at 1% concentration as indigestible marker for determination of minerals digestibility. From the mineral digestibility results, it was revealed that the highest digestibility values (%) of Ca, Na, K and P were observed at2 gKg^{-1} level of probiotics supplementation and these values differed significantly ($p<0.05$) from the control diet and other test diets. Hence, it was concluded that probiotics supplementation in MOSM based diets at 2 gKg^{-1} level is enough to release sufficient amount chelated minerals for improving overall performance of *L. rohita* fingerlings.

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Introduction

Aquaculture is the fastest growing food producing industry in the world. The main objective of global aquaculture is to maximize the efficiency of food production system (Dey *et al.*, 2015). The expected fish production will be up to 53.64 million metric tonnes in 2030. An extra demand of food fish will be 30-40 million tonnes to fulfill the increasing need for growing population in 2050 (Amarasinghe *et al.,*2016). Fish is performing a central role in food security and poverty control (Fielder *et al.*, 2016). It contains high concentrations of essential fatty acids, minerals, vitamins and protein (Wheal *et al.*, 2016). It is also unique source of iron (Fe), zinc (Zn), and omega-3 fatty acids (Kunsch *et al.*, 2012).

Labeo rohita is widely considered to be one of the most important Indian major carp (Hussain *et al.,* 2015). It is column feeder and has high commercial value due to consumer's choice in subcontinent like Pakistan and India. It has economic and ecological importance and can be kept easilyin laboratory conditions (Balasubramanian *et al.*, 2016). The fish feed industry depends on the use of fishmeal (FM) as a major source of nutrients such as amino acids, fatty acids, vitamins, major minerals and other growth factors (Zhou *et al.*, 2004). However, excessive use of fishmeal in aqua-feed is promoting a negative impact on water bodies by liberating more amounts of nitrogen (N) as well as phosphorous (P) in aquatic environment (Cheng and Hardy, 2002). The second most important issue is increasing fishmeal prices due to restricted supply, which motivated the researchers to identify the cost effective alternatives of fishmeal (Barnes *et al.*, 2012; Lech and Reigh, 2012; Dedeke *et al.*, 2013; Shapawi *et al.*, 2013). Plant by-products are one of the best source of protein and energy for the formulation of environment friendly as well as economical aqua-feeds (Cheng and Hardy, 2002; Gatlin *et al.*, 2007).

Two species of family Moringaceae are reported in Pakistan, of which one is *Moringa oleifera* (Manzoor *et al.*, 2007).

M. oleifera seed meal has been used as source of the major feed ingredient as it contains high quality protein, fats and major minerals (Compaore *et al.*, 2011) and. It is easily available and cost effective as compared to other oilseed meals. It also contains active coagulating agents characterized as dimeric cationic proteins, having molecular weight of 13 kilo Dalton (k Da) and anti-microbial activities (Anwar *et al.*, 2007). Sulfur (S) containing amino acids (methionine, tryptophan and cystine) are also present in it (Makkarand Becker, 1996). It has a potential to become an important fish feed ingredient to improve fish growth due to its high nutritional values (Yuagsoi and Masamoto, 2014).

Probiotics are defined as non-digestible food supplements (Gibson, 2004). Majority of these probiotics are nonpathogenic, non-toxic and can survive in the gut and remain potent for long period under storage and field condition (Ramakrishnan *et al.*, 2008). Probiotic colonization enhanced diet-dependent absorption of Fe and Zn (Ahire *et al.*, 2018). Nowadays, probiotics are becoming an integral part of the aquaculture practices to obtain high production (Kumar *et al.*, 2016), due to involvement of probiotics in nutrition and other beneficial activities in fish has proven beyond doubt. Probiotics can help to build up the beneficial bacterial flora in the gut and completely or partially exclude the pathogenic bacteria. In view of the above mentioned facts and in continuation of our work on formulation of fish feed by probiotics supplementation may be expected to provide economic and environment friendly benefits through decreased supplemental mineral as well as mineral discharge in aquatics environment.

Materials and methods

The present research work was conducted to evaluate the effects of probiotics (Protexin®) supplementation onmineral digestibility of *L. rohita* fingerlings fed on MOSM based diets. Experiment was carried out in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad.

Experimental design

Plant by-products such as MOSM was used as test Each experimental diet was then divided into seven test diets and supplemented with graded levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 gkg^{-1}) of probiotics (Protexin®). One control diet and six probiotics supplemented MOSM based test diets were fed to seven fish groups stocked in water tanks. Duration of the each trial was 90-days. Each oilseed meal based diet supplemented with probiotics was compared with each other and control diet to determine mineral digestibility by using Completely Randomized Design (CRD). The relationships between mineral digestibility and probiotics supplementation levels were established by using quadratic regression analysis by using IBM SPSS statistics-23 software.

Fish and experimental conditions

L. rohita fingerlings were purchased from Government Fish Seed Hatchery, Faisalabad. Fingerlings were bathed in 0.5% saline solution for 1 to 2 minutes to make sure that they were free from ectoparasites and to prevent from fungal infection (Rowland and Ingram, 1991). Prior to trial, fingerlings were allowed to acclimatize for two weeks in particularly designed V-shape water tanks, having 70 L water capacity. During this period, the fingerlings were fed once daily on basal diet (Allan and Rowland, 1992). The air pump was used to supply fresh air by capillary system through-out the experimental period.

Study of water quality parameters

Water quality parameters such as pH, temperature and dissolved oxygen were monitored through pH meter (Jenway3510) and DO meter (Jenway970) on daily basis. The range of water quality parameters such as temperature was 25.2-29.1°C, pH 7.6-8.7, dissolved oxygen 5.7-7.5 mgL^{-1} and electrical conductivity 1.30-1.52 dSm^{-1}.

Formation of feed pellets

The feed ingredients including MOSM were obtained from local fish feed market. The seeds were air-dried and defatted by press method (Salem and Makkar, 2009). Processed MOSM were finely grinded to pass through 0.3 mm sieve size.

Prior to the formulation of the experimental diets, the feed ingredients were chemically analyzed (Table 1) by standard methods (AOAC, 1995). An inert marker, chromic oxide (Cr_2O_3) was added at the rate of 1% in all test diets. While, Ingredients composition (%) of oilseeds meals based diets supplemented with probiotics was shown in table-2.Feed ingredients were mixed slowly into the electric mixer after adding 10-15% of tap water, resulting in suitably textured dough and were processed through pelleting machine to formulate pellets (Lovell, 1989). Fish oil was also steadily added during this mixing process. One control and six (total seven test diets) were formulated by adding graded levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 gKg^{-1}) of probiotics (Protexin® $CFUg^{-1}$ = 2×10^9; A multi-strains Pobiotics Animal Nutrition, Probiotics International Ltd, England). All experimental diets were dried under shady place and stored at 4°C until use.

Feeding protocol and sample collection

Fish fingerlings were fed at the rate of 4 % of live wet weight on their prescribed diets twice (8:00 am and 4:00 pm) a day. After the feeding session of two hours, the unutilized diet was drained out from each tank by opening the valves of tanks. The tanks were washed to remove the feed particles and refilled with fresh tap water. Feces were collected by the opening valve-I and valve-II subsequently from the fecal collecting tube of each replicated tank. Fecal material was collected carefully to avoid the breakage of feces for minimizing the leaching of nutrients especially calcium and phosphorus in water. The uneaten diet and feces were dried in oven at 65ºC and stored for further chemical analysis.

Estimation of minerals

For estimation of minerals, the diets and feces samples were digested in a boiling nitric acid and perchloric acid mixture (2:1) according to AOAC, (2005). After appropriate dilution, mineral contents were estimated by using Atomic Absorption Spectrophotometer. The estimation of sodium (Na) and potassium (K) was performed through flame photometer (Jenway PFP-7, UK). Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent by using UV-VIS 2001 Spectrophotometer at 370 nm absorbance (Divakaran *et al*., 2002). The apparent digestibility of nutrients i.e. crude protein, crude fat, apparent gross energy, calcium (Ca), sodium (Na), potassium (K), copper (Cu), iron (Fe), phosphorous (P), magnesium (Mg) and aluminium (Al) of test diets was determined indirectly at the end of the experiment using chromic oxide as inert marker. The estimation of Na and K was done through flame photometer (Jenway PFP-7, UK). Phosphorus was analyzed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as reagent at 720 nm absorbance by standard methods (AOAC, 2005).

Calculation of apparent digestibility coefficient (ADC)

Apparent minerals digestibility coefficients (ADC) of test diets were calculated by the formula reported in NRC (1993).

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

Statistical analysis

Finally, data of minerals (Ca, Na, K, Fe, Cu, P, Mg and Al) were subjected to one-way Analysis of Variance (Steel *et al*., 1996). The differences among means of treatments were compared by Tukey's Honesty Significant Difference Test and considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The CoStat Computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis. IBM SPSS statistical software-23 was used for graphical presentation.

Results

Feces excreted by *L. rohita* fingerlings showed that there was a significant ($p<0.05$) difference among the amount of minerals when fish fed on probiotics supplemented MOSM based test diets and control diet. Overall results indicated that highest amount of minerals was discharged through feces in water when *L. rohita* fingerlings fed on control diet (0 gKg^{-1}). Lowest minerals discharge (Ca, Na, K and P) was observed by fish fed on 2 gKg^{-1} level. While other minerals (Fe and Cu) excretion through feces was noted lowest when fish fed on 1.5 gKg^{-1} level based diet (Table 3). It was also observed that mineral discharge by fish fed on probiotics supplemented diets was significantly ($p<0.05$) different from minerals discharged by fish fed on control diet. Quadratic regression analysis indicated that optimum Ca, Na, K, Mg, P, Fe and Al in feces of *L. rohita* fed probiotics supplemented MOSM based test diets occurred at 1.75, 2.04, 1.93, 2.22, 1.94, 1.81 and 1.97 gKg^{-1} level, respectively (Fig. 1-8).

Table 1. Chemical composition (%) of feed ingredients (Dry matter basis).

Ingredients	Dry matter (%)	Gross Energy (kcal/g)	Crude Protein (%)	Crude Fat(%)	Crude Fiber (%)	Ash (%)	Total Carbohydrates (%)
Fish meal	92.49	3.79	45.27	8.58	1.63	21.51	23.01
Wheat flour	91.54	2.74	9.89	2.23	2.81	3.25	81.82
Corn gluten 60%	91.99	4.19	59.12	5.96	2.36	2.22	30.34
Rice polish	93.89	4.21	13.54	10.25	3.89	6.89	65.43
MOSM*	93.09	3.69	37.09	6.63	2.58	6.24	47.46

*MOSM= *M. oleifera* seed meal.

Table 4showed that minerals digestibility such as Ca (55%), Na (54%), K (47%), Fe (46%), Cu (49%),Mg (53%) and Al (49%) were less in fingerlings fed on control diet (0gKg^{-1}) followed by fish fed on test diet II.

The mineral digestibility started increasing from 0.5gKg^{-1} level and reached its maximum at 2 gKg^{-1} level followed by fish fed on 2.5 gKg^{-1} level based diet. Whereas, the maximum digestibility of Fe (69%) and Cu (66%) was found at 1.5 gKg^{-1} level based diet.

Maximum digestibility of Ca (74%), Na (75%), K (74%) and P (75%) were found at 2 gKg^{-1} level followed by fish fed on 2.5 gKg^{-1} level based diet. While the maximum mineral (Mg and Al) digestibility was observed at 2.5 gK^{-1} level followed by 2 gK^{-1} level based diet. It was also observed that further increase in probiotics supplementation ppt 03 gKg^{-1} level, did not increased digestibility of minerals.

On the other side Mg (64%) and Al (64%) was maximally absorbed when fish fed on 2.5gKg^{-1} level that were statistically at par (63 and 62%, respectively) with the values found from fish fed on 2 gKg^{-1} level based diet (Table 4). These values were statistically ($p<0.05$) different when compared with the digestibility values found in fish fed on control diet.

Table 2. Ingredients composition (%) of oilseeds meals based diets supplemented with probiotics (As fed basis).

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI	Test Diet-VII
Probiotics (gKg^{-1}) Protexin*	0	0.5	1	1.5	2	2.5	3
MOSM	50	50	50	50	50	50	50
Fish meal	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Wheat flour	12	11.5	11	10.5	10	9.5	9
Rice polish	12	12	12	12	12	12	12
Fish oil	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Vitamin Premix	1	1	1	1	1	1	1
Mineral Premix	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1

*Probiotics were used at the expense of wheat flour

Protexin* consist of *Lactobacillus plantarum, L. bulgaricus, L. acidophillus, L. mamophilus, Bifidobacterium bifidum, Streptococcus faecium, Aspergillus oryzae* and *Candida printolopesi* ($CFUg^{-1} = 2\times10^9$).

Table 3. Analyzed mineral composition (%) in feces of *L. rohita*fed MOSM based control diet and other test diets supplemented with probiotics.

Diets		Ca	Na	K	P	Fe	Cu	Mg	Al
Probiotics Levels (gKg^{-1})	0 (Control diet) (Test diet I)	0.510^{a}	0.0075^{a}	0.687^{a}	0.967^{ab}	0.033^{a}	0.0035^{a}	0.0052^{ab}	0.0032^{a}
	0.5 (Test diet II)	0.419^{bc}	0.0070^{a}	0.636^{ab}	1.044^{a}	0.028^{ab}	0.0033^{a}	0.0055^{a}	0.0030^{ab}
	1 (Test diet III)	0.376^{cd}	0.0062^{a}	0.582^{b}	0.940^{b}	0.024^{bc}	0.0025^{c}	0.0049^{ab}	0.0028^{ab}
	1.5 (Test diet IV)	0.359^{d}	0.0058^{a}	0.475^{c}	0.761^{c}	0.018^{d}	0.0023^{c}	0.0045^{bc}	0.0027^{ab}
	2 (Test diet V)	0.286^{e}	0.0043^{a}	0.336^{e}	0.560^{d}	0.019^{d}	0.0026^{bc}	0.0041^{c}	0.0025^{b}
	2.5 (Test diet VI)	0.324^{de}	0.0049^{a}	0.394^{d}	0.678^{c}	0.021^{cd}	0.0032^{ab}	0.0041^{c}	0.0024^{b}
	3 (Test diet VII)	0.447^{b}	0.0062^{a}	0.593^{b}	0.941^{b}	0.026^{bc}	0.0035^{a}	0.0048^{abc}	0.0029^{ab}
PSE		0.01193636	0.0008766	0.01198416	0.01839837	0.00112094	0.00012435	0.00015157	0.00013657
p-value		0.0000	0.2100	0.0000	0.0000	0.0000	0.0000	0.0001	0.0078

Means Within rows having different superscripts are significantly different at P<0.05

Data are means of three replicates

PSE =Pooled SE = √MSE/n (where MSE =mean-squared error.

It was concluded that digestibility of most of the minerals were started to increase from 0.5 gKg^{-1} level till 2 gKg^{-1} level based diet. On the other hand it steadily decreased up to 3 gKg^{-1} level of probiotics supplementation.

Quadratic regression analysis indicated that optimum Ca, Na, K, Mg, P, Fe and Al digestibility for *L. rohita* fed probiotics supplemented MOSM based test diets occurred at 1.74, 2.17, 1.93, 1.92, 1.79, 2.21 and 2.00 gKg^{-1} level, respectively (Fig. 9-16). From these results it was concluded that 2 gKg^{-1} level based diet is most optimum for maximizing the mineral digestibility and reducing water pollution.

Table 4. Apparent mineral digestibility (%) of *L. rohita* fingerlings fed MOSM based control diet and other test diets supplemented with probiotics

Diets		Ca	Na	K	P	Fe	Cu	Mg	Al
Probiotics Levels (gKg^{-1})	0 (Control diet) (Test diet I)	54.62^{f}	53.59^{e}	47.55^{f}	58.44^{d}	46.12^{e}	48.80^{e}	53.42^{d}	48.56^{e}
	0.5 (Test diet II)	62.57^{d}	58.46^{d}	51.82^{e}	55.40^{e}	54.48^{d}	53.28^{c}	51.39^{d}	53.21^{d}
	1 (Test diet III)	66.70^{c}	62.69^{c}	56.44^{d}	60.30^{d}	60.34^{c}	64.32^{b}	56.52^{c}	56.75^{bc}
	1.5 (Test diet IV)	68.22^{c}	66.60^{b}	64.50^{c}	67.43^{c}	69.39^{a}	66.52^{a}	59.39^{b}	58.60^{b}
	2 (Test diet V)	74.47^{a}	75.38^{a}	74.50^{a}	75.70^{a}	68.49^{a}	62.73^{b}	62.58^{a}	61.69^{a}
	2.5 (Test diet VI)	71.42^{b}	73.22^{a}	70.30^{b}	70.68^{b}	64.42^{b}	54.70^{c}	63.61^{a}	63.59^{a}
	3 (Test diet VII)	59.72^{e}	65.38^{b}	55.61^{d}	59.57^{d}	56.57^{d}	51.02^{d}	56.95^{bc}	55.60^{c}
PSE		0.49063126	0.49003401	0.47009456	0.48557444	0.49368553	0.43825594	0.5141243	0.46049662
p-value		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Means Within rows having different superscripts are significantly different at $P<0.05$

Data are means of three replicates

PSE =Pooled SE = $\sqrt{MSE/n}$ (where MSE =mean-squared error.

Discussion

In the present study, probiotics supplementation at the level of 2 gKg^{-1} efficiently increased mineral contents in *L. rohita* fingerlings, when fed MOSM based diets.

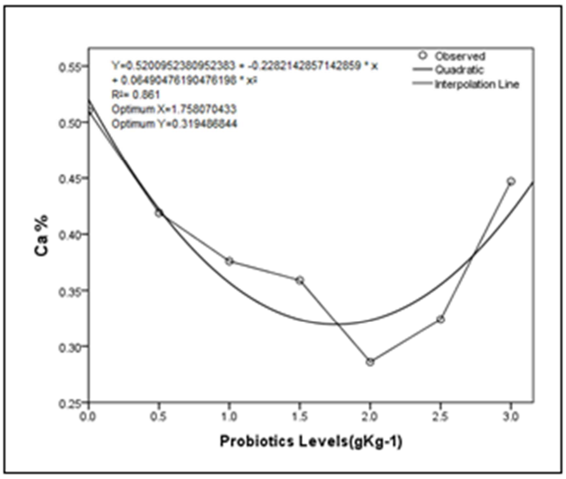

Fig. 1. Quadratic relationship between Ca (%) in feces of *L. rohita* fingerlings and MOSM based test dietssupplemented with probiotics

It is well known that phytate commonly present in plant based ingredients usually binds with divalent cations and is known as a major anti-nutritional factor (Soetan and Oyewole, 2009). Nwanna *et al.* (2007) reported that phytate binds with major minerals, makes them unavailable to fish and reduce their digestibility.

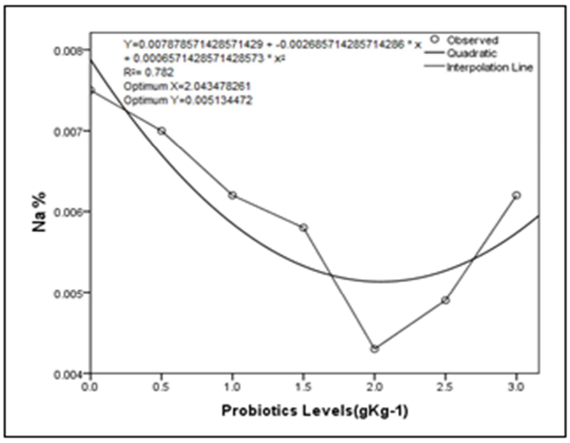

Fig.2. Quadratic relationship between Na (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics

Overall current results indicated that highest amount of minerals (Ca, Na, K, P, Cu, Mg and Al) was discharged through feces in water when*L. rohita* fingerlings fed on control diet (0 gKg^{-1}).Many other researchers also confirmed that phytate present in plant by-products may chelate with some of the important minerals i.e. Na, Fe, Ca, P, Mn, Cu, Ni, Cr, K and Mg (Cao *et al.*, 2007; Hussain *et al.*, 2014, 2015,c; Hussain *et al.*, 2015,d).

Lopez *et al.* (2000) also reported the availability of minerals such as Zn, Fe, Cu, and Ca are depressed by phytate. Probiotics bacteria produce phytase enzyme, which can release minerals depressed by phytate, resulting in increased availability of minerals (Parvaneh *et al.,* 2014, Lan *et al.*, 2002).

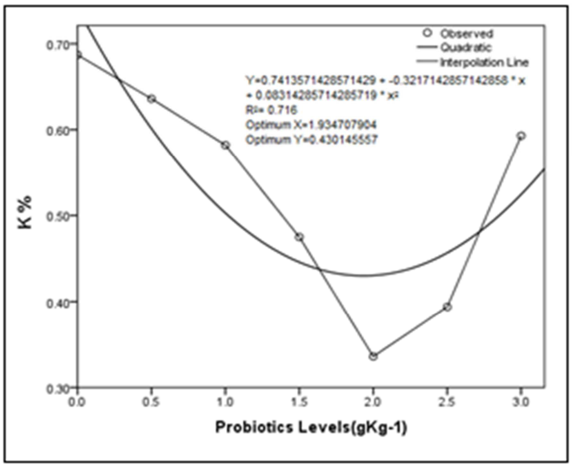

Fig. 3. Quadratic relationship between K (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

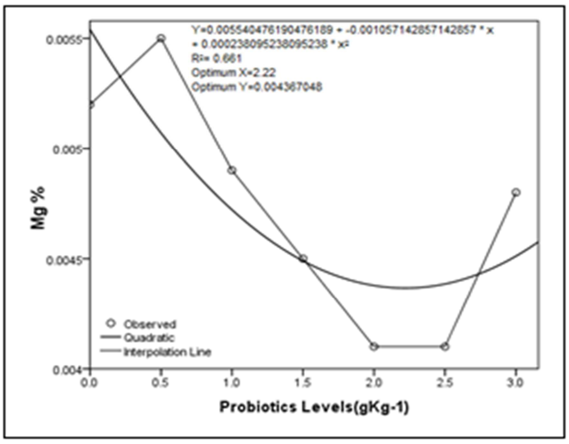

Fig. 4. Quadratic relationship between Mg (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics

So the breakdown of phytate may improve release of essential nutrients especially minerals. Dan *et al.* (2017) reported two phytase-producing bacterial strains, (*Staphylococcus caprae* CCF2 and *Bacillus cereus* LRF5) isolated from the gut of *C. catla and L. rohita*, respectively were used in soybean meal based diet and these strains were effective in reducing the anti-nutritional factors, phytic acid and enhancing protein, lipid as well as mineral concentration.

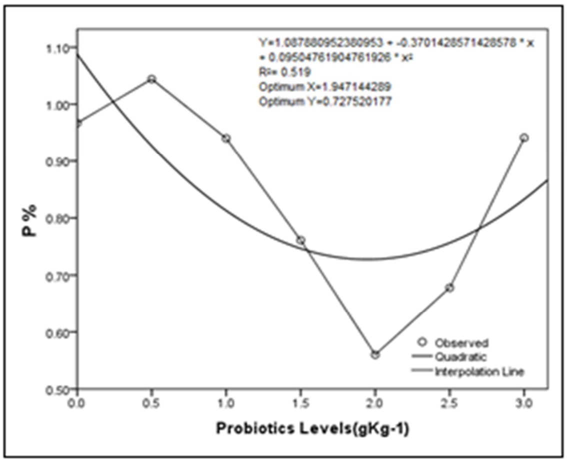

Fig. 5. Quadratic relationship between P (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

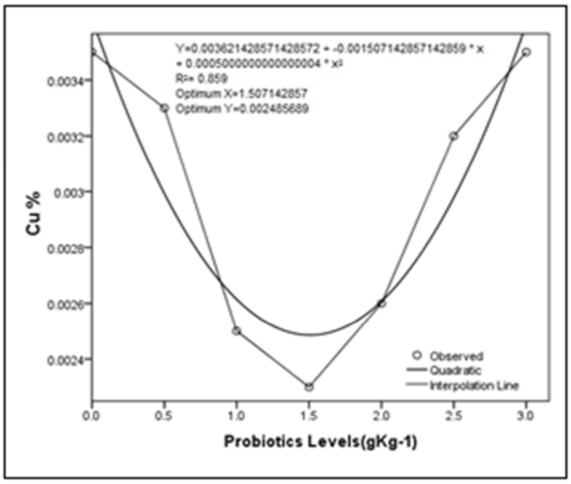

Fig. 6. Quadratic relationship between Cu (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

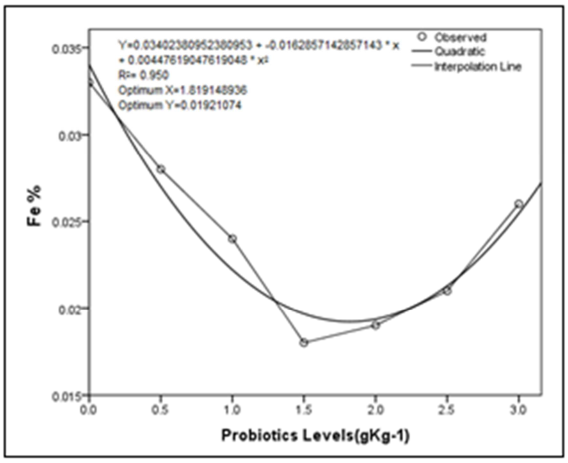

Fig.7. Quadratic relationship between Fe (%) in feces of *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

Present results also indicated that supplementation of probiotics in MOSM based diets is much beneficial for mineral digestibility in *L. rohita* fingerlings as compared to fish fed on control diet, because of the possible breakdown of chelated minerals phytate complex.

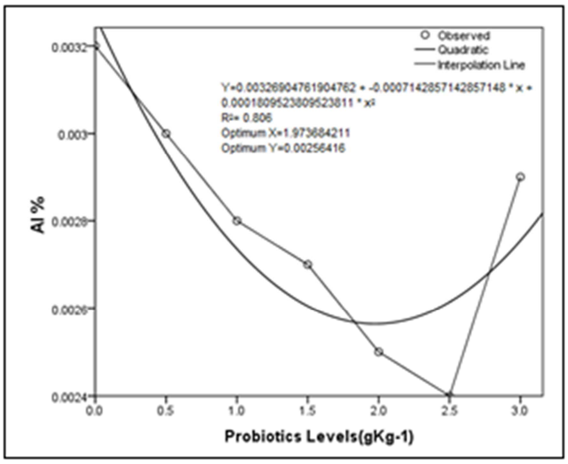

Fig. 8. Quadratic relationship between Al (%) in feces of *L.* rohita fingerlings and MOSM based test diets supplemented with probiotics.

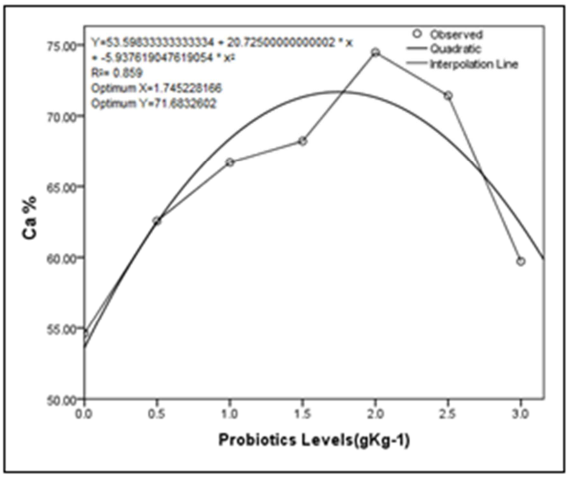

Fig. 9. Quadratic relationship betweenCa (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

One of the most important effects of probiotics on bone possibly occurs via synthesis of vitamins (Hancock and Viola, 2001). Vitamins such as C, D, K, and folate are involved in metabolism of Ca and are important for bone formation (Weber, 1999; Parvaneh *et al.*, 2014). From present research, it was observed that 2 gKg^{-1} are the most optimum levels of probiotics supplementation that can reduce excretion of these essential minerals in water through feces of *L. rohita* fingerlings. Highest mineral digestibility by *L. rohita* fingerlings was noted when fed 2 gKg^{-1} probiotics supplemented MOSM based diets. This value was statistically ($p<0.05$) different when compared with the digestibility values found in fish fed on control diet.

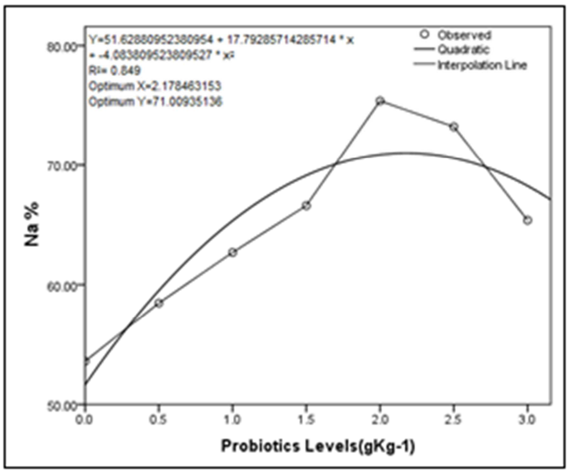

Fig. 10. Quadratic relationship between Na (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

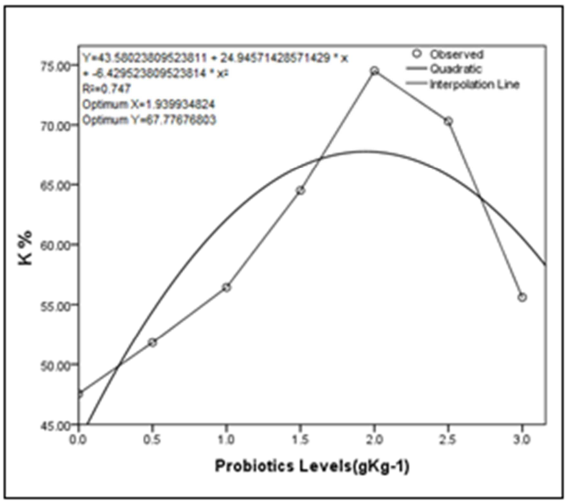

Fig. 11. Quadratic relationship between K (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

It confirms that probiotics supplementation is most effective way for improving mineral's digestibility and decreasing the nutrients discharge through feces into water, resulting in minimized water pollution.

Rodrigues *et al.* (2012) also confirmed that mineral contents such as Ca, P and Mg were absorbed maximum in animals when fed with probiotics (*Lactobacillus and Bifidobacterium*)supplemented yacon flour.

Digestibility of minerals such as calcium (Ca), sodium (Na), potassium (K), phosphorous (P), iron (Fe), copper (Cu), magnesium (Mg) and aluminum (Al) showed significant difference among different probiotics levels fed to Indian major carps fingerlings.

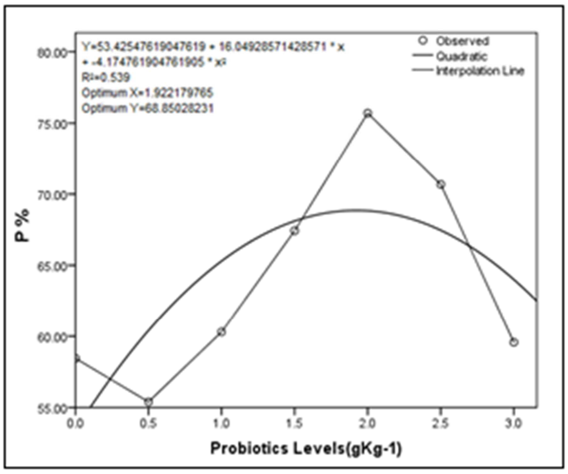

Fig.12. Quadratic relationship between P (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

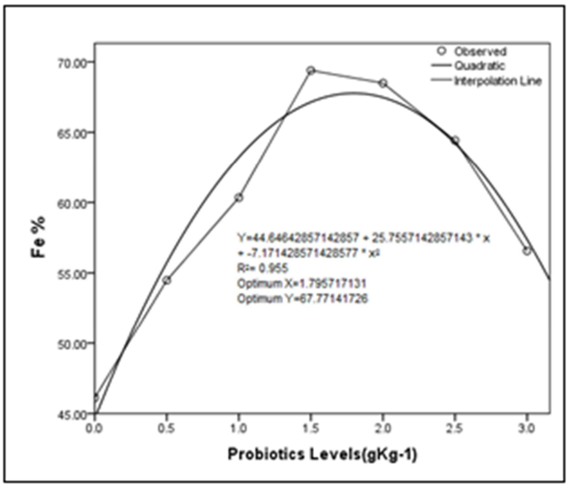

Fig. 13. Quadratic relationship between Cu (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

It appears that probiotics provided benefits by establishing favorable microbial communities such as *Bacillus* sp. and lactic acid bacteria (LAB) in the gastrointestinal track which may alter gut morphology, produce certain enzymes, inhibitory compounds causing improved digestion as well as digestibility of minerals (Verschuere *et al.*, 2000).

Most of bacteria produce short chain fatty acids followed by an improvement in mineral digestibility via their solubility (Campbell *et al.*, 1997).

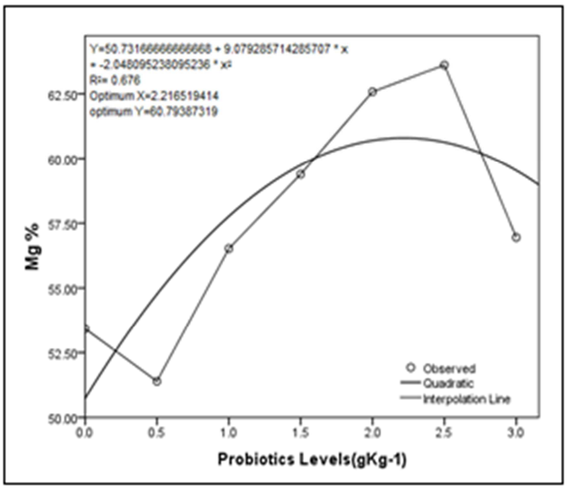

Fig. 14. Quadratic relationship between Mg (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

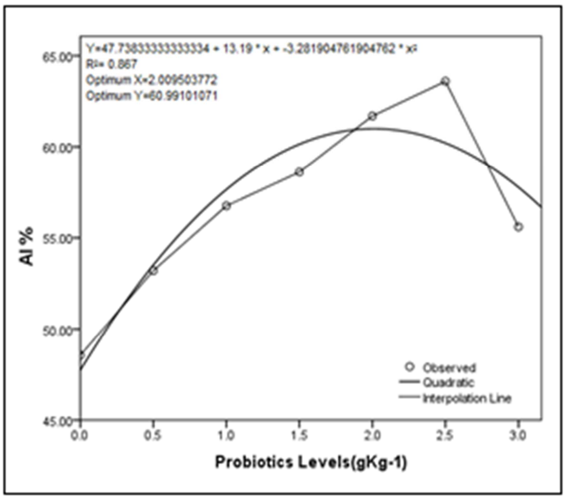

Fig.15. Quadratic relationship between Al (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

The optimum level of probiotics (2 gKg^{-1}) in MOSM based diet improved mineral digestibility in *L. rohita* fingerlings. It may be due positive activities of probiotics by reducing intestinal inflammation and production of short chain fatty acids followed by increasing bone mass density (Parvaneh *et al.*, 2014).

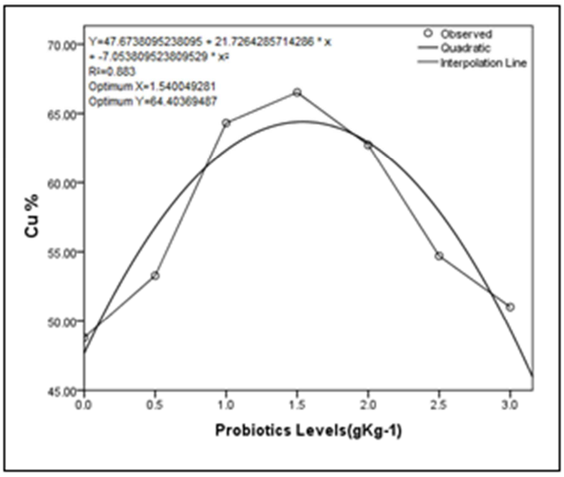

Fig. 16. Quadratic relationship between Cu (%) digestibility for *L. rohita* fingerlings and MOSM based test diets supplemented with probiotics.

Conclusion

In conclusion, the present study provided sufficient evidences that 2 gKg^{-1} level of probiotics supplementation had a significant effect on the mineral digestibility of *L. rohita* fingerlings fed on MOSM based diets without compromising growth. Increased use of naturally occurring minerals in plant ingredients reduces the need for mineral supplements in formulated diets and results in decreased elimination of minerals in feces. Thus, preparation of fish feed by probiotics supplementation may be expected to provide both economic as well as environmental benefits through decreased expenditures on supplemental minerals and mineral discharged to the aquatic ecosystem.

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