Effectiveness of phytase pre-treatment on growth performance, nutrient digestibility and mineral status of common carp (Cyprinus carpio) juveniles fed Moringa by-product based diet

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Several anti-nutritional substances are found in plant derivatives for example phytate, that make the nutrients and minerals unavailable to fish, hence leading to poor growth performance. Presence of the anti-nutrient factor such as phytate is a chelated compound and need enzyme for its breakdown and availability of nutrients to improve fish growth. This research work was performed to check the improvement of overall performance of Cyprinus carpio fingerlings by the help of phytase addition in Moringa oleifera by-products based diet. Combination of Moringa seed meal and Moringa leaf meal was utilized as test ingredient to formulate seven test feeds, containing graded levels of phytase (0, 500, 650, 800, 950, 1100 and 1250 FTU kg⁻¹). In feeding trial of 70 days, fingerlings were given feed two times in a day at the rate of 4% of wet weight of their bodies and faeces were collected. According to current results, it was found that growth performance parameters i.e. weight gain; 25 g, specific growth rate; 1.67 and feed conversion ratio; 1.10 were improved to maximum at 950 FTU kg⁻¹. Digestibility of nutrients (crude protein; 73%, crude fat; 71% and gross energy; 67%) and minerals absorption was also maximum (Ca; 70%, Zn; 66%, K; 74%, Mn; 66% and P; 71%) at 950 FTU kg⁻¹. Lowest growth efficiency, nutrient digestibility and mineral absorption were observed in fingerlings fed at control diet (0 FTU kg⁻¹). Results of the current study, proved that 950 FTU kg⁻¹ is the most optimum level of phytase to formulate economical and eco-friendly feed for improved growth of C. carpio fingerlings as it decreases the discharge of minerals and nutrients in water bodies.

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1. Introduction

The fastest and developing source of animal protein is aquaculture, providing almost half of the entire fish consumed worldwide (Bostock et al., 2010). On account of the rapid human population rise and to fulfill the need of quality food, aquaculture industry has been developed as rapidly growing food producing sector (Ibrahem et al., 2010). In the past, fishmeal (FM) was used as chief source of protein in fish food (Shahzad et al., 2017), because of the presence of large amount of essential nutrients for example amino acids, lipids, growth factors and vitamins, high digestibility and low level of carbohydrate (Zhou et al., 2004; Morales et al., 2014). But, shortage and high cost of FM leads it to a need of using low cost alternate protein sources that are plant derived (Morales et al., 2014). Various plant by-products based diets such as corn gluten (Hussain et al., 2015a; Hussain et al., 2018), sunflower (Rabia et al., 2017), soybean meal (Hussain et al., 2015b; Chu et al., 2016), Moringa oleifera seed meal (Shahzad et al., 2018a)
and *Moringa oleifera* leaf meal (Shahzad et al., 2017) were given to different fish species. One of the economical alternative source of plant protein is *Moringa oleifera*, commonly recognized as “sohan-jana” in Pakistan and easily available in southern Punjab (Hassan et al., 2018). MOLM (*M. oleifera* leaf meal) was utilized as feed for common carp (Yuangsoi and Masumoto, 2012), Nile tilapia (Afuang et al., 2003; Richter et al., 2003), African catfish (Samkelisiwe and Ngonidzashe, 2014) and *Catla catla* (Shahzad et al., 2017), because it contains higher protein content ranging from 25 (Makkar and Becker, 1996) to 32% (Soliva et al., 2005). MOSM (*M. oleifera* seed meal) contains rich amount of protein (32–35%), essential vitamins, amino acids (Hassan et al., 2018) and minerals i.e. P, Na, K, Ca, Zn and Mg etc. (Anjorin et al., 2010).

But major problem in utilization of plant by-product based protein sources such as *M. oleifera* is the presence of several antinutritional factors (Danwitz et al., 2016). Most important antinutritional factor among these is phytic acid (PA) or phytate (Reddy, 2002). In salt form, phytate comprises 60 to 80% of entire P (phosphorus), chelated form of magnesium, calcium and sodium salts found in plant based diets (Lei et al., 2013). It acts as a lock around P and bounds with other minerals such as Mg, Mn, Cu, Fe and Ca (Denstadli et al., 2006) and decreases their absorption (Hussain et al., 2011a; Dawood et al., 2015; Hussain et al., 2015b). It results in adverse effects on growth performance of monogastric and agastic fishes (NRC, 1993).

Due to these harmful effects, there is a need of breakdown of phyate-mineral complexes present in plant by-product based diets. Phytic acid cannot be removed easily unless some enzymatic reactions are performed (Vielma et al., 2000). Phytase (PHY) enzyme is very effective to catalyze the hydrolysis of PA and extracts P, making it available for absorption (Kumar et al., 2012a). PHY removes phosphate from PA in a stepwise process (Vielma et al., 2000). Phytase (PHY) enzyme is very effective to catalyze the hydrolysis of PA and extracts P, making it available for absorption (Kumar et al., 2012a). PHY removes phosphate from PA in a stepwise process (Vielma et al., 2000) and decreases their absorption (Hussain et al., 2011a; Dawood et al., 2015; Hussain et al., 2015b). It results in adverse effects on growth performance of monogastric and agastic fishes (NRC, 1993).

2. Materials and methods

The current experimental work was performed to check the improvement of overall performance of common carp fingerlings, using Moringa derivatives, supplemented with PHY to estimate its impact on growth, nutrient digestibility and mineral absorption. The experiment was carried out in Zoology Department, Division of Science and Technology, University of Education, Lahore.

2.1. Fish and experimental conditions

Common carp fingerlings were procured from Sindwa Fish Hatchery, Head Balloki, Kasur. Before starting experiment, the cars were acclimatized for almost ten days and were kept (fifteen fingerlings in each) in water tanks of V shape with 70 L water holding capacity. During entire period, PHY supplemented moringa derivatives based-feed was given twice a day (Allan and Rowland, 1992). Parameters related to water quality for example temperature, dissolved oxygen and pH were checked regularly and air pump was used for proper supply of oxygen by capillary system. Before starting the feed trial, cars were treated with saline solution (0.5%) for 1–2 min to eliminate the pathogens (Rowland and Ingram, 1991).

2.2. Experimental strategy

Derivatives of moringa plant (combination of MOSM and MOLM) were used as test ingredient and mixture of feed was distributed into seven test feeds, sprayed with varied amounts (0, 500, 650, 800, 950, 1100 and 1250 FTU kg⁻¹) of PHY enzyme. Out of these seven test diets, one control (0 FTU kg⁻¹) and six PHY supplemented test diets were given to seven groups of fingerlings. They were fed at the rate of 4% of live wet body weight for 70 days. Each test and control diet were compared with each other to identify growth, mineral absorption and nutrient digestibility.

2.3. Processing of Moringa derivatives

*M. oleifera* seeds were purchased from local market of Lahore. Seeds were first dried in air and then de-fatted using press method (Salem and Makkar, 2009). Fresh leaves of moringa were taken from botanical garden of University of Education, Lahore and then washed to make them clean and dirt-free. These leaves were dried under a shady place for nearly six days to protect the vitamins from harm via photo-dynamic oxidation. Dry leaves were detached from the stems to reduce amount of fibre in feed (Madalla et al., 2013). Treated Moringa leaves and seeds were crushed to convert them into powder. The remaining feed components were obtained from market and were ground to pass them through 0.3 mm sieve size. Before the formulation of experimental diets, chemical composition of all diet components was studied by using subsequent standard methods (AOAC, 1995). Firstly, in all experimental diets, 1% Cr₂O₃ (as an inert marker) was used and mixed with all other components of feed carefully for at least 5–10 min. Then, slow blending of components was done after the addition of 10 to 15% distilled water, to make properly textured dough. Processing of dough was done within the pelleting machine to form pellets (Lovell, 1989). Moringa derivatives were used to prepare six PHY added and one control test diet. whereas, 50 mL distilled water was used to prepare the various concentrations of PHY enzyme (0, 500, 650, 800, 950, 1100 and 1250 FTU kg⁻¹) and were sprayed on every test diet (Robinson et al., 2002). Similar amount of distilled water was also sprayed on control (0 FTU kg⁻¹) diet to maintain the moisture. At the end, all the prepared diets were dried out and then stored at almost 4 °C till use (see Table 1).

2.4. Feeding procedure and assemblage of sample

*C. carpio* fingerlings were given prescribed feed two times daily, and after completion of feeding time (two hours), the leftover diet was removed from each tank. The tanks were washed properly to eliminate the particles of feed and were refilled. Faeces were collected from the faecal accumulating tube of every tank by opening the valve–1 and 2 subsequently. Faecal material was collected carefully to lessen the faeces breakage so that nutrients are not leached.
out in water and were dried in oven at 65 °C for 3 to 4 h and then stored for further analysis.

2.5. Growth study

To study the growth of fingerlings, fifteen carps of average weight (5.65 g fish\(^{-1}\)) were kept in each tank and were bulk weighed on periodical basis throughout the experimental period to assess the growth efficiency of *C. carpio*. Growth parameters for example FCR, weight gain (%) and specific growth rate (SGR) of carps were calculated by using standard formulae (NRC, 1993).

Weight gain\(\%\) = \[
\frac{\text{Final weight (w₂) } - \text{Initial weight (w₁)} \times 100}{\text{Initial weight}}
\] (1)

SGR\(\%\) = \[
\frac{(\ln \text{final wt. of fish} - \ln \text{initial wt. of fish}) \times 100}{\text{Test days}}
\] (2)

FCR = \[
\frac{\text{Total dry feed intake (g)}}{\text{Wet WG (g)}}
\] (3)

2.6. Chemical analysis of faeces and feed

After 70 days of feeding period, homogenized samples of diet and faeces were dried in oven at 105 °C for almost 12 h and moistness was measured. CP (crude protein) contents were identified by using Micro Kjeldahl Apparatus, whereas EE (ether extract) was analysed through Soxhlet system, by using petroleum ether extraction method. GE (gross energy) was estimated by Oxygen bomb calorimeter. Following formula was used to calculate total carbohydrate contents.

Total carbohydrates\(\%\) = \[
\frac{(\text{Moisture} \% + \text{CP} \% + \text{CF} \% + \text{EE} \% + \text{Ash} \%)}{100}
\]

2.7. Assessment of minerals

To estimate the minerals, Atomic Absorption Spectrophotometer, was used according to AOAC (1995) and potassium and sodium were estimated by flame photometer. Contents of marker present in faeces and diets were assessed after oxidation with molybdate reagent (Divakaran et al., 2002) and UV–VIS 2001 Spectrophotometer was used at absorbance of 370 nm. Contents of phosphorous in the diets and faeces were determined with the help of UV/VIS spectrophotometer at absorbance of 720 nm.

2.8. Calculation of ADC (\%)

Apparent digestibility coefficient (ADC) of nutrients present in diets was measured by using the following formula (NRC, 1993);

\[
\text{ADC (\%) } = \frac{\text{marker in feed (\%)} \times \text{nutrients in faeces (\%)}}{\text{marker in faeces (\%)} \times \text{nutrients in feed (\%)}} - 100
\] (4)

2.9. Statistical analysis

Finally, data of ADC\(\%\) of nutrients (GE, EE and CP), minerals (Ca, K, Na, Fe, P, Cu, Al, Mg, Mn and Zn) and growth was analyzed by one-way ANOVA test. The differences among all treatments were compared using Tukey's Honesty Significant Difference Test by considering significant at \(p < 0.05\) (Biswas et al., 2019). The CoStat Computer Package was used to perform statistical analysis.
3. Results

3.1. Growth study of C. carpio

Table 2 presents the mean values of total growth performance i.e. WG (g), WG (%), FCR, SGR and survival rate of C. carpio, feeding on MOSM + MOLM combination based test diets. According to our results, C. carpio that were given 950 FTU kg⁻¹ PHY level based diet, showed maximum weight gain (19 g), WG (350%), SGR (1.67) and minimum FCR (1.10), showing highest conversion of feed into weight and ultimately decreases the water pollution.

| Test diets | PHY levels (FTU kg⁻¹) | Initial weight | PW | WG% | WG / fish / day | Feed intake | FCR | SGR | Survival rate |
|------------|-----------------------|----------------|-----|-----|-----------------|-------------|-----|-----|--------------|
| Diet-I     | 0                     | 5.66 ± 0.31    | 18.41 ± 0.64² | 12.75 ± 0.36² | 225.71 ± 7.90² | 0.18 ± 0.01² | 0.28 ± 0.02² | 1.52 ± 0.06² | 1.31 ± 0.03² | 91.79 ± 3.72 |
| Diet-II    | 500                   | 5.62 ± 0.25    | 20.82 ± 0.64⁴ | 15.19 ± 0.41⁴ | 270.35 ± 6.72⁴ | 0.22 ± 0.01⁴ | 0.31 ± 0.01⁴ | 1.43 ± 0.04⁴ | 1.45 ± 0.02⁴ | 94.00 ± 5.89 |
| Diet-III   | 650                   | 5.66 ± 0.19    | 22.29 ± 0.46² | 16.63 ± 0.28² | 293.69 ± 4.76² | 0.24 ± 0.00² | 0.31 ± 0.01² | 1.31 ± 0.03² | 1.52 ± 0.01² | 95.96 ± 3.51 |
| Diet-IV    | 800                   | 5.64 ± 0.25    | 24.17 ± 0.72² | 18.49 ± 0.47² | 327.82 ± 6.02² | 0.26 ± 0.01² | 0.32 ± 0.01² | 1.20 ± 0.03² | 1.61 ± 0.02² | 97.92 ± 3.61 |
| Diet-V     | 850                   | 5.63 ± 0.29    | 25.38 ± 0.97² | 19.74 ± 0.70² | 350.69 ± 7.64² | 0.28 ± 0.01² | 0.31 ± 0.01² | 1.10 ± 0.02² | 1.67 ± 0.02² | 97.92 ± 3.61 |
| Diet-VI    | 1100                  | 5.65 ± 0.16    | 23.67 ± 0.56³ | 16.02 ± 0.34³ | 318.99 ± 3.31³ | 0.26 ± 0.00² | 0.34 ± 0.01² | 1.32 ± 0.03³ | 1.59 ± 0.01³ | 95.96 ± 3.51 |
| Diet-VII   | 1250                  | 5.64 ± 0.17    | 20.48 ± 0.42³ | 14.84 ± 0.25³ | 263.13 ± 3.64³ | 0.21 ± 0.00² | 0.32 ± 0.01² | 1.50 ± 0.05³ | 1.43 ± 0.01³ | 91.79 ± 3.72 |

³Means within column having dissimilar superscripts are quietly different at p < 0.05. Data are means of three replicates with fifteen fingerlings in each.
fore, PHY should always be added in diet in view of all these factors (Cao et al., 2007b). In current research, it was explained that 950 FTU kg\(^{-1}\) is best PHY level for \(C.\) carpio to increase the digestibility of CP, EE and GE. Similar to these results, Shahzad et al. (2018a; 2020) found highest digestibility of crude protein and fat in \(C.\) catla fed on 900 FTU kg\(^{-1}\) in MOSM and \(M.\) oleifera by-products based diets respectively. They also noticed that further increment in PHY dose (upto the level of 1500 FTU kg\(^{-1}\)) does not increase the digestibility of CP, EE and GE. Similarly, Maas et al. (2018) observed highest CP digestibility in Nile tilapia, and maximum value of fat digestibility (80%) was observed in rohu by Bano and Afzal (2017) when given SBM containing 1000 FTU kg\(^{-1}\). Little different results were shown in research work of Thanh et al. (2017) and Hussain et al. (2011b, 2011c; 2015d), who observed maximum

| Test diets  | PHY level (FTU kg\(^{-1}\)) | CP (%) | EE (%) | GE (%) |
|------------|-----------------------------|--------|--------|--------|
| Diet –I (Control) | 0 | 52.69 ± 0.83\(^{e}\) | 50.42 ± 0.78\(^{d}\) | 48.66 ± 0.97\(^{d}\) |
| Diet –II | 500 | 60.28 ± 0.90\(^{c}\) | 58.44 ± 0.94\(^{c}\) | 57.33 ± 0.96\(^{c}\) |
| Diet –III | 650 | 66.48 ± 0.91\(^{b}\) | 63.26 ± 0.98\(^{b}\) | 61.69 ± 0.75\(^{b}\) |
| Diet –IV | 800 | 71.51 ± 0.94\(^{a}\) | 69.73 ± 0.65\(^{a}\) | 66.33 ± 0.64\(^{a}\) |
| Diet –V | 950 | 73.35 ± 0.89\(^{a}\) | 71.60 ± 0.77\(^{a}\) | 67.66 ± 0.91\(^{a}\) |
| Diet –VI | 1100 | 67.55 ± 0.93\(^{b}\) | 64.69 ± 0.86\(^{b}\) | 63.69 ± 0.90\(^{b}\) |
| Diet –VII | 1250 | 57.35 ± 0.90\(^{d}\) | 56.44 ± 0.58\(^{c}\) | 57.69 ± 0.98\(^{c}\) |

\(^{a-f}\)Means within column having dissimilar superscripts are quietly different at p < 0.05. Data are means of three replicates with fifteen fingerlings in each.
Table 4
Examined composition of minerals in a diet, containing mixture of MOSM + MOLM.

| Test diets | PHY level | Ca  | Na  | K   | P   | Fe  | Cu  | Mg  | Al  | Zn  | Mn  |
|------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Diet-I (Control) | 0  | 0.86 ± 0.08 | 0.017 ± 0.002 | 1.28 ± 0.07 | 2.08 ± 0.08 | 0.051 ± 0.007 | 0.0059 ± 0.0008 | 0.0094 ± 0.0007 | 0.00050 ± 0.00008 | 0.039 ± 0.008 | 0.021 ± 0.007 |
| Diet-II    | 500 | 0.86 ± 0.06 | 0.016 ± 0.002 | 1.28 ± 0.08 | 2.08 ± 0.07 | 0.051 ± 0.007 | 0.0059 ± 0.0009 | 0.0093 ± 0.0006 | 0.00051 ± 0.0004 | 0.038 ± 0.003 | 0.020 ± 0.009 |
| Diet-III   | 650 | 0.85 ± 0.05 | 0.018 ± 0.002 | 1.28 ± 0.05 | 2.08 ± 0.10 | 0.051 ± 0.008 | 0.0058 ± 0.0006 | 0.0094 ± 0.0007 | 0.00052 ± 0.0007 | 0.038 ± 0.003 | 0.021 ± 0.008 |
| Diet-IV    | 800 | 0.85 ± 0.06 | 0.017 ± 0.004 | 1.28 ± 0.05 | 2.09 ± 0.08 | 0.050 ± 0.010 | 0.0059 ± 0.0008 | 0.0093 ± 0.0008 | 0.00052 ± 0.0007 | 0.039 ± 0.008 | 0.022 ± 0.006 |
| Diet-V     | 950 | 0.85 ± 0.09 | 0.017 ± 0.004 | 1.27 ± 0.08 | 2.08 ± 0.05 | 0.050 ± 0.008 | 0.0059 ± 0.0007 | 0.0094 ± 0.0009 | 0.00052 ± 0.0010 | 0.039 ± 0.006 | 0.021 ± 0.004 |
| Diet-VI    | 1100 | 0.87 ± 0.06 | 0.018 ± 0.003 | 1.29 ± 0.09 | 2.08 ± 0.09 | 0.050 ± 0.006 | 0.0060 ± 0.0007 | 0.0093 ± 0.0007 | 0.00051 ± 0.0009 | 0.040 ± 0.004 | 0.022 ± 0.003 |
| Diet-VII   | 1250 | 0.87 ± 0.08 | 0.017 ± 0.005 | 1.28 ± 0.05 | 2.07 ± 0.07 | 0.052 ± 0.009 | 0.0059 ± 0.0009 | 0.0095 ± 0.0007 | 0.00050 ± 0.0005 | 0.040 ± 0.008 | 0.022 ± 0.006 |

Data are means of three replicates with fifteen fingerlings in each.

Table 5
Examined composition of minerals in C. carpio faeces fed on diet containing mixture of MOSM + MOLM.

| Test feeds | PHY levels | Ca  | Na  | K   | P   | Fe  | Cu  | Mg  | Al  | Zn  | Mn  |
|------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Diet-I (Control) | 0  | 0.55 ± 0.05a | 0.007 ± 0.001a | 0.62 ± 0.02a | 1.13 ± 0.06a | 0.029 ± 0.004a | 0.0031 ± 0.0005a | 0.0051 ± 0.0003a | 0.00028 ± 0.00005ab | 0.023 ± 0.004a | 0.012 ± 0.004a |
| Diet-II    | 500 | 0.47 ± 0.04a | 0.006 ± 0.000a | 0.57 ± 0.04a | 0.99 ± 0.02ab | 0.023 ± 0.002ab | 0.0027 ± 0.0005ab | 0.0043 ± 0.0003abc | 0.00022 ± 0.0002ab | 0.019 ± 0.001ab | 0.009 ± 0.004a |
| Diet-III   | 650 | 0.40 ± 0.03bc | 0.007 ± 0.001a | 0.48 ± 0.02b | 0.79 ± 0.04b | 0.020 ± 0.003ab | 0.0024 ± 0.0003ab | 0.0037 ± 0.0002cd | 0.00019 ± 0.00003a | 0.017 ± 0.001ab | 0.009 ± 0.003a |
| Diet-IV    | 800 | 0.34 ± 0.03cd | 0.006 ± 0.001a | 0.43 ± 0.01bc | 0.72 ± 0.04cd | 0.015 ± 0.004b | 0.0022 ± 0.0003ab | 0.0033 ± 0.0004cd | 0.00021 ± 0.00003ab | 0.015 ± 0.003ab | 0.008 ± 0.002a |
| Diet-V     | 950 | 0.28 ± 0.02cd | 0.006 ± 0.001a | 0.36 ± 0.01bc | 0.66 ± 0.05c | 0.017 ± 0.003bc | 0.0021 ± 0.0002ab | 0.0036 ± 0.0004cd | 0.00024 ± 0.00004ab | 0.015 ± 0.003ab | 0.008 ± 0.002a |
| Diet-VI    | 1100 | 0.36 ± 0.02cd | 0.008 ± 0.001a | 0.43 ± 0.04bc | 0.76 ± 0.03cd | 0.022 ± 0.003ab | 0.0020 ± 0.0003ab | 0.0039 ± 0.0003bcd | 0.00027 ± 0.00004ab | 0.015 ± 0.002b | 0.009 ± 0.001a |
| Diet-VII   | 1250 | 0.44 ± 0.04bc | 0.009 ± 0.002a | 0.55 ± 0.02a | 0.92 ± 0.02ab | 0.028 ± 0.005a | 0.0028 ± 0.0004ab | 0.0047 ± 0.0009ab | 0.00032 ± 0.00002a | 0.019 ± 0.003ab | 0.011 ± 0.003a |

Means within column having dissimilar superscripts are quietly different at p < 0.05.
Data are means of three replicates with fifteen fingerlings in each.
Examined minerals absorption in feeding on diet containing mixture of MOSM + MOLM. *C. carpio*

| Test feeds | PHY levels | Ca  | Na  | K  | P  | Fe  | Cu  | Mg  | Al  | Zn  | Mn  |
|------------|------------|-----|-----|----|----|-----|-----|-----|-----|-----|-----|
| Diet-I (Control) | 0           | 40.73 ± 0.90e | 60.43 ± 0.98c | 55.75 ± 0.92e | 50.20 ± 0.92f | 47.56 ± 0.85f | 51.81 ± 0.90e | 50.44 ± 0.90f | 48.36 ± 0.96f | 45.20 ± 0.55f | 45.30 ± 0.90f |
| Diet-II | 500         | 50.44 ± 0.63d | 64.29 ± 0.82b | 59.22 ± 0.88d | 56.12 ± 0.33e | 58.46 ± 0.93d | 57.63 ± 0.93d | 57.41 ± 0.82d | 60.73 ± 0.88c | 53.34 ± 0.75e | 60.56 ± 0.82d |
| Diet-III | 650         | 57.27 ± 0.88c | 66.22 ± 0.96b | 65.62 ± 0.62c | 65.41 ± 0.79c | 64.53 ± 0.90c | 62.31 ± 0.96c | 64.23 ± 0.98b | 66.35 ± 0.81a | 59.64 ± 0.78c | 61.38 ± 0.67cd |
| Diet-IV | 800         | 63.53 ± 0.90b | 69.41 ± 0.87a | 69.59 ± 0.91b | 68.35 ± 0.99b | 71.65 ± 0.95a | 65.73 ± 0.86b | 67.37 ± 0.90a | 63.47 ± 0.59b | 64.16 ± 1.08b | 65.20 ± 0.76ab |
| Diet-V | 950         | 70.25 ± 0.99a | 65.36 ± 0.71b | 74.50 ± 0.71a | 71.35 ± 0.86a | 68.64 ± 0.87b | 67.67 ± 0.85ab | 65.55 ± 0.82ab | 57.69 ± 0.79d | 66.34 ± 0.86ab | 66.37 ± 0.79a |
| Diet-VII | 1250       | 53.85 ± 0.84c | 52.54 ± 0.98d | 60.65 ± 0.95d | 59.58 ± 0.93d | 51.32 ± 0.96e | 56.32 ± 0.87d | 54.47 ± 0.83e | 42.40 ± 0.94g | 56.73 ± 0.88d | 52.28 ± 0.84e |

$p < 0.05$. Data are means of three replicates with fifteen fingerlings in each.

High levels of PHY i.e. 1000, 2000 and 4000 FTU kg\(^{-1}\) of PHY increased digestibility of P and retention of Cu at 500 to 950 FTU kg\(^{-1}\), while increase in level of PHY (1100 and 1250 FTU kg\(^{-1}\)) did not increase the absorption coefficient of minerals (Shahzad et al., 2020). Value of minerals absorption was found to be lowest at control diet. Close to the outcomes of current study, Shahzad et al. (2018a and b) found higher absorption and bioavailability of minerals in *C. catla* fed on 900 FTU kg\(^{-1}\) added in MOSM and mixture of MOLM and MOSM based diets, respectively. Similarly, Maas et al. (2018) and Rabia et al. (2017) observed highest absorption of few minerals in Nile tilapia and *L. rohita* respectively at 1000 FTU kg\(^{-1}\). Little different results were shown in research work of Hussain et al. (2016) and Marjan et al. (2014). They observed highest digestibility of minerals in *L. rohita* when given feed containing 750 FTU kg\(^{-1}\) of PHY. More different from our results were those of Qu and Davis, (2016). They noticed improved digestibility of P and retention of Cu at 500 and 2000 FTU kg\(^{-1}\) in *Litopenaeus vannamei*. High levels of PHY i.e. 1000, 2000 and 4000 FTU kg\(^{-1}\) gave enhanced P digestibility, bone minerals and reduced faecal P in *Clarias gariepinus* (Olugbenga et al., 2017). In contrast to present findings, Nwanna et al. (2005) observed better Ca deposition and fewer P discharge at 8000 FTU kg\(^{-1}\) in *C. gariepinus*. Contrarily, negative impacts were also observed regarding minerals absorption e.g. in research work of Xue (2014). They observed no difference in retention of minerals excluding Zinc on feeding PHY supplemented SPC based diet to Nile tilapia in comparison of the control diet. In the light of above-mentioned results, we can argue that the possible explanations for dissimilarities in results of minerals retention and absorption may be the quantity, quality (Baruah et al., 2007b; Dersjant-Li et al., 2015) and dissimilar sources of PHY, feed components, methods of diet formulation or changed fish species (Liu et al., 2013).
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