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Phytase Enzyme Ameliorates Growth Performance, Mineral Digestibility, Amino Acid Digestibility and Body Chemical Composition of the Common Carp (*Cyprinus carpio* L.) at Rearing Stage

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ABSTRACT

Phytase enzyme supplementation in feed is expected to increase the bioavailability of phosphorus and other nutrients. The present study aimed to evaluate the effect of phytase enzyme supplementation in feed on growth, mineral digestibility, amino acid digestibility and body chemical composition of common carp (*Cyprinus carpio* L.) fed with soybean meal at the rearing stage. The study used common carp fish with an average weight of 18.82 ± 0.26 g/head. The experimental feed was floating pellets supplemented with various doses of phytase enzyme: 0 (A), 500 (B), 1,000 (C) and 1,500 (D) FTU/kg feed. Mineral content (Ca, Mg, Fe, Cu, Zn and Mn) was analyzed from the dried fish feces using a polarized atomic absorption spectrophotometer. While Na and K contents were determined using a flame photometer, and P content was calorimetrically analyzed. The feed utilization efficiency (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), survival rate (SR) and protein digestibility (ADCp) were determined. The results showed that common carp fed with 1000 FTU/kg phytase enzyme (C) exhibited the highest PER, FCR, EFU and RGR values, compared to other experimental feeds. Common carps given 1000 FTU/kg phytase enzyme (C) recorded the highest mineral values, including Ca, P, Na, K, Mg, Fe, Cu, Mn and Zn. Therefore, phytase enzyme supplementation in feed significantly (P<0.05) increased the growth, mineral digestibility, protein content and amino acid digestibility of *C. carpio*. The phytase enzyme at 1000 FTU/kg of feed was the best dose for common carp at the rearing stage.

INTRODUCTION

Feed is a radical factor determining the success of intensive fish farming **(Hussain *et al*., 2014)**. For most fish farmers, especially common carp farmers, the high feed cost,

around 60-70% of the total production cost, is a major problem. This is because the price of fish meals is increasingly high, thus encouraging the search for alternative raw materials to replace cost-effective fish meals **(Bello *et al*., 2012; Shapawi *et al*., 2013)**. Soybean meal flour can be used as an alternative protein source to replace fish meals because it is cost-effective and good for fish feed **(Khan *et al*., 2011; Nahashon & Kilonzo-Nthenge, 2013; Hussain *et al*., 2015a; Hussain *et al*., 2015b)**. However, soybean meal contains anti-nutritional substances such as phytic acid, which reduces the nutritional quality of the feed because it interferes with the absorption and digestion of fish **(Kumar *et al*., 2012)**.

Phytic acid can form phytic-protein and phytic-mineral-protein complexes, causing digestive problems **(Laining *et al.*, 2010)**. In addition, phytic acid can also bind to amino acids, thereby reducing the availability of amino acids **(Banaee *et al*., 2013)**. Due to their inability to produce phytase in their digestive systems, monogastric and agastric fish cannot hydrolyze the phytic acid content. Common carp are monogastric fish that cannot consume phosphorus bounded from phytate due to a lack of phytase enzymes in their digestive tract **(Liebert & Portz, 2005)**. Thus, the phytate-bound phosphorus will be excreted into the water, causing water pollution and increasing eutrophication **(Vats *et al*., 2005)**. Phosphorus is an important building block of nucleic acids and cell membranes, a key structural component of tissues, and is directly involved in all energy-producing processes **(National Research Council [NRC], 2011)**. Lack of phosphorus in feed reduces growth and feed conversion **(Zhu *et al.*, 2014)**.

One strategy to solve the phytic acid problem is by supplementing the phytase enzyme in fish feed to increase the digestibility of protein and minerals **(Liu *et al*., 2013)**. Phytase enzyme supplementation in the feed will hydrolyze phytic acid, releasing phosphorus from the phytate complex. Therefore, phosphorus freely available in the feed plays an important role in improving the overall performance of fish **(Pham *et al*., 2008)**, as well as increasing the bioavailability of minerals for fish, making it cost-effective and environmentally friendly **(Gabriel *et al*., 2007; Hussain *et al*., 2011a, Hussain *et al*., 2014)**. When phytase enzymes are added to fish feed, the fish utilize phosphorus and enhance nutrient bioavailability **(Olusola & Nwanna, 2014; Hussain *et al*., 2015a; Hussain *et al*., 2015b)**. Furthermore, the supplementation of phytase enzymes in the feed increases the absorption of minerals such as Ca, P, Cu, Mg, Sr, Zn and Fe **(Caipang *et al*., 2011)**.

Information about phytase supplementation in soybean meal-based feed as a source of vegetable protein is still lacking in common carp at the rearing stage, thus this research is necessary. Therefore, the present study aimed to evaluate the effect of phytase supplementation in feed on growth, mineral digestibility, amino acid digestibility and body chemical composition of grow-out stage common carp fed soybean meal as a source of vegetable protein.

MATERIALS AND METHODS

Experimental fish and research design

The experimental fish were 300 common carp fish at the rearing stage, with an average weight of 18.82 ± 0.26 g/head. The fish were obtained from the Balai Benih Ikan (BBI), Sawangan, Magelang, Central Java, Indonesia. Before the experiment, fish were

adapted for one week. During adaptation, fish specimens were given artificial feed without phytase enzyme supplementation using *ad satiation* method 3 times a day. The fish were selected according to **Rachmawati *et al.* (2017)**. Fish were not disabled, uniform in size and weight, actively swimming and healthy. The experimental fish fasted a day before the study to clean up the remaining feed given earlier. At the beginning of the study, the fish with known initial weight were inserted into a fiber tub (1x1x1 m^3) filled with 30 liters of water (1 fish/liter). Experimental feeds were given to the fish with 5% fixed feeding rate three times a day at 07.00 am, 13.00 pm, and 19.00 pm. Fish were weighed every week during the 63- day study. Siphoning was done to maintain the feasibility of water quality for fish by using a hose to remove feces and leftover feed in the bottom of the fiber tub. Siphoning was done approximately 2 h after feeding. Water quality parameters were observed, including pH (6.5-8.6), DO (≥3 mg/L) and temperature (25-30°C), using a digital water quality meter (AZ8603, China); the ammonia content was determined using ammonia medium range checker (Hanna Instrument HI715, Indonesia). All water quality parameters were determined according to the method of **Boyd (2003)**.

Experimental feed preparation

The study used four experimental feeds in the form of floating pellets supplemented with phytase enzyme at the dose of 0 (A), 500 (B), 1,000 (C), and 1,500 (D) FTU/kg feed and 1% chromic oxide (Cr_2O_3) as an indicator of protein digestibility **(National Research Council [NRC], 2011)**. The phytase enzyme (Nathupos® E 10000 G) was produced by BASF SE, Ludwigshafen, Germany. The experimental feed was formulated according to the feed formulation (Table 1). Experimental fish consisted of fish meal (animal protein source), soybean meal (vegetable protein source), corn flour, bran flour and tapioca flour (carbohydrates source), fish oil and oil corn (fat source), vitamin-mineral mix (vitamins and minerals source), carboxymethyl cellulose binder/CMC (binder), phytase enzymes and Cr_2O_3 (digestibility indicator). Next, all ingredients were mixed except for fat and water that were added after the ingredients were mixed **(National Research Council [NRC], 2011)**. The feed mixture was stirred until becoming homogeneous using a mixer. The fish oil, corn oil and water were then mixed until getting remarkably homogeneous. The homogeneous feed mixture was put into the extruder floating pellet molding machine (H 2700, China). The feed was then air-dried at 26°C. Furthermore, the dried feed was packaged in airtight plastic and stored until further use.

Research container

Twelve fiber tubs (1x1x1 m^3) were used as research containers. Each fiber tub was equipped with a recirculation system to stabilize water quality to remain within the optimum range. The maintenance media used a water source precipitated in the reservoir.

Collection of fish feces and mineral analysis

Common carp feces from each treatment with three replications (12 experimental units) were collected. Feeding method was a fixed feeding rate of 5%/weight of biomass, with a frequency of feeding 2 times a day (morning and evening). Two hours after the feeding time, the uneaten feed was collected, and the remaining feed was removed by removing the media water from each research container by opening the fiber tub valve.

Furthermore, the fiber tub was filled again with water as much as the volume of water was removed.

Table 1. Experimental feed formulation (100 g)

Ingredient (g)	**Treatment**			
	A (0 FTU/kg feed)	**B (500 FTU/kg feed)**	**C (1,000 FTU/kg feed)**	**D (1,500 FTU/kg feed)**
Fish flour	15.00	15.00	15.00	15.00
Soybean meal	26.00	26.00	26.00	26.00
Corn Eggplant	24.20	24.19	24.18	24.17
Bran	21.30	21.30	21.30	21.30
Tapioca flour	4.50	4.50	4.50	4.50
Fish oil	1.00	1.00	1.00	1.00
Corn oil	1.50	1.50	1.50	1.50
Mineral vitamin[1)]	4.00	4.00	4.00	4.00
Phytase enzyme (FTU)	0.00	0.01	0.02	0.03
Carboxymethyl cellulose binder (CMC)	1.50	1.50	1.50	1.50
Cr_2O_3	1.00	1.00	1.00	1.00
Total	100	100	100	100
Proximate analysis result				
Protein (%)*	30.16	30.10	30.18	30.15
Fat (%) *	8.54	9.20	9.02	9.18
Non-nitrogen free extract (NNFE) (%)*	32.18	33.09	33.05	32.16
Energy (kkal)[2)]	249.28	249.34	249.42	249.28
Ratio E/P (kal/g)[3)]	8.85	8.87	8.86	8.89

Note:

[1)] Vitamin and Mineral mix kg^{-1} : magnesium (Mg) 1,900 mg, Vit. B2 97 mg, Vit. B6 46 mg, potassium (K) 150 mg, calcium (Ca) 219 mg, sodium (Na) 117 mg, selenium (se) 150 mg, Vit. B1 52 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, Vit. B12 60 mg, vitamin A 36,000 IU, vitamin D3 9,000 IU, manganese (Mn) 105 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, vitamin C (coated) 68,800 mg activity, Zinc (Zn) 90 mg, Pantothenic acid 93 mg, Vit. K3 19 mg, Niacin 130 mg., Folic acid 10 mg, Inositol 225 mg, Biotin 450 mg, Vit. E 187 mg.

[2)] The digestible energy according to 1g of protein is 3.5 kcal/g, 1 g of fat is 8.1 kcal/g, and 1 g of carbohydrates is 2.5 kcal/g **(National Research Council [NRC], 2011)**.

[3)] E/P value for optimal fish growth ranges from 8-12 kcal/g **(National Research Council [NRC], 2011)**.

* Proximate analysis was done in the Animal Feed Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University.

Mineral analysis was done by drying fish feces from each treatment in an oven and grinding and storing them for chemical analysis. The mineral content of the test feed and fish feces was estimated by diluting in a boiling mixture of nitric acid and perchloric acid (2:1) **(Association of Official Analytical Chemists [AOAC], 2005)**. After dilution, mineral content (Ca, Mg, Fe, Cu, Zn and Mn) was determined using a polarized atomic absorption spectrophotometer (Hitachi Z-8200, Jjepang). Mineral estimation was evaluated using commercially available standard solutions (AppliChem® Gmbh

Ottoweg4, DE-64291 Darmstadt, Jerman). Na and K values were analyzed using a flame photometer (Jenway PFP-7, UK). While, P wascalorimetrically analyzed (UV/VIS spectrophotometer), using ammonium molybdate reagent at 720 nm **(Association of Official Analytical Chemists [AOAC], 2005)**.

Minerals digestibility

Chromic oxide (Cr_2O_3) was used as an indirect indicator of feed digestibility with the assumption that the amount of Cr_2O_3 in the feed and feces remains constant during the experimental period, and that all Cr_2O_3 eaten in the fish feed appears in the feces **(National Research Council [NRC], 2011)**. The amount of Cr_2O_3 in feed and feces was estimated using a UV-VIS 2001 spectrophotometer at an absorbance of 370nm after being oxidized with a molybdate reagent **(Divakaran *et al*., 2002)**. The digestibility of minerals such as Ca, P, Mg, Na, K, Fe, Cu, Zn, and Mn was determined indirectly at the end of the experiment using Cr_2O_3 as a digestibility marker. The nutrient digestibility coefficient (ADC) of the test feed was calculated using the method of the **National Research Council [NRC] (2011)**.

Proximate analysis of test feed and fish carcass

Proximate analysis refers to the **National Research Council [NRC] (2011)** method. Protein content was determined using a semi-automated Kjeldahl system (FOSS Kjeltec 2300). Fat content was determined using the ether extraction method based on the Soxhlet method (FOSS Soxtec 2043). Ash content was determined by burning test feed samples and fish in a furnace at 550°C for 24h.

Observed parameters

The feed utilization efficiency (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), survival rate (SR) and protein digestibility (ADCp) were determined according to the **National Research Council [NRC] (2011)** using the following formula:

$$ADCp\ (\%) = 100 - \left\{\frac{100 \times Cr_2O_3\ (feed)}{\%\ Cr_2O_3\ (feces)} \times \frac{\%\ protein\ (feces)}{\%\ protein\ (feed}\right\}$$

$$EFU\ (\%) = \frac{final\ weight - initial\ weight}{Feed\ consumed\ weight} \times 100$$

$$RGR\ (\%) = 100 \times \frac{final\ weight - initial\ weight}{experimental\ times\ \times initial\ weight}$$

$$FCR = \frac{feed\ intake\ (g)}{body\ weight\ gain\ (g)}$$

$$PER = 100 \times \frac{final\ weight - initial\ weight}{Diet\ consumed\ amount \times protein\ content\ in\ diet}$$

$$SR\ (\%) = 100 \times \frac{final\ count}{initial\ count}$$

Statistical analysis

Growth observation and amino acid digestibility data were analyzed using one-way analysis of variance (ANOVA) with $P<0.05$. Duncan tests were then performed to

determine the difference in mean values between treatments **(Steel *et al.*, 1996)**. All data were analyzed using SAS software V9.3 (SAS Institute, Cary, NC, USA).

RESULTS

The common carp fish had higher EFU, ADCp, PER, FCR and RGR values after being fed with experimental feed containing phytase enzyme, compared to that without phytase enzyme (Table 2). Common carp fed with 1000 FTU/kg phytase enzyme (C) exhibited the highest PER, FCR, EFU and RGR values, compared to other experimental feeds.

Tabel 2. The initial fish weight (IW), final fish weight (FW), weight gain (WG), feed utilization efficiency (EFU), protein digestibility (ADCp), protein efficiency ratio (PER), feed conversion ratio (FCR), relative growth rate (RGR) and survival (SR) of common carp during the study

Parameter	Experimental feed			
	A (0 FTU/kg feed)	B (500 FTU/kg feed)	C (1,000 FTU/kg feed)	D (1,500 FTU/kg feed)
IW (g)	18.82 ± 0.26	18.78 ± 0.24	18.90 ± 0.28	18.78 ± 0.26
FW (g)	68.25 ± 0.38^{d}	97.82 ± 0.34^{b}	106.07 ± 0.32^{a}	89.15 ± 0.22^{c}
WG (g)	49.43 ± 0.32^{d}	79.04 ± 0.30^{b}	87.17 ± 0.30^{a}	70.37 ± 0.23^{c}
ADCp (%)	59.17±0.26^{d}	67.20±0.34^{b}	78.62±0.32^{a}	64.48±0.15^{c}
EFU (%)	58.36±0.12^{d}	66.40±0.20^{b}	76.58±0.23^{a}	62.24±0.21^{c}
PER	1.79±0.24^{d}	2.56±0.22^{b}	3.24±0.27^{a}	2.32±0.25^{c}
FCR	1.76±0.20^{d}	1.38±0.26^{b}	1.05±0.29^{a}	1.45±0.23^{c}
RGR (%/day)	2.00±0.22^{d}	2.56±0.27^{b}	3.14±0.23^{a}	2.28±0.23^{c}
SR (%)	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}

Note: Values with the same superscript in the row showed no significant difference (P>0.05).

The effect of the experimental feed on the chemical composition of the common carp body is shown in Table (3). The highest crude protein and ash content were observed on phytase enzyme treatment. However, the treatments had no significant (P>0.05) differences in crude fat and dry matter.

Table 3. Body chemical composition of common carp fed experimental feed

Treatment	Dry matter	Protein	Lipid	Ash
1	25.56±1.22^{a}	54.62±0.28^{b}	25.59±1.16^{a}	18.50±0.35^{b}
2	24.98±0.74^{a}	56.30±0.67^{a}	24.89±0.62^{a}	20.07±0.71^{a}
3	24.24±0.99^{a}	56.69±0.53^{a}	24.47±0.70^{a}	20.12±0.33^{a}
4	25.83±0.67^{a}	57.35±0.58^{a}	25.33±0.63^{a}	19.68±0.87^{a}
5	24.43±0.67^{a}	56.89±1.09^{a}	24.96±1.38^{a}	19.77±0.49^{a}

Note: Different superscripts in the same column indicated significantly different (P<0.05).

Mineral digestibility in the body and feces of common carp fed with experimental feed are presented in Tables (4, 5). The common carp specimens given 1000 FTU/kg

phytase enzyme (C) had the highest minerals, including Ca, P, Na, K, Mg, Fe, Cu, Mn and Zn.

Table 4. Digestibility of minerals (%) in common carp fed different doses of phytase enzyme

Mineral	Experimental feed			
	A (0 FTU/kg feed)	B (500 FTU/kg feed)	C (1000 FTU/kg feed)	D (1500 FTU/kg feed)
Ca	53.12±2.13^{d}	63.05±3.04^{c}	71.22±2.34^{a}	59.48±2.36^{b}
P	45.27±2.54^{d}	59.32±2.48^{c}	77.16±2.40^{a}	65.75±2.26^{b}
Na	49.16±2.20^{d}	52.24±2.19^{c}	69.11±2.85^{a}	58.72±3.08^{b}
K	52.32±2.18^{d}	60.40±2.31^{c}	73.33±2.41^{a}	66.19±1.50^{b}
Mg	40.22±1.34^{d}	52.13±1.02^{c}	64.26±0.12^{a}	60.24±2.31^{b}
Fe	49.46±2.18^{d}	54.07±0.34^{c}	68.39±2.46^{a}	59.24±1.09^{b}
Cu	55.22±2.45^{d}	60.32±2.51^{c}	69.02±1.17^{a}	64.20±1.16^{b}
Mn	50.46±0.13^{d}	57.26±2.49^{c}	69.35±2.54^{a}	60.31±0.12^{b}
Zn	59.21±0.21^{d}	64.31±1.40^{c}	73.26±1.40^{a}	69.48±0.72^{b}

Note: Different superscripts in the row indicated significantly different (P<0.05).

Table 5. Digestibility of minerals (%) in feces of common carp fed on different doses of phytase enzyme

Mineral	Experimental feed			
	A (0 FTU/kg feed)	B (500 FTU/kg feed)	C (1000 FTU/kg feed)	D (1500 FTU/kg feed)
Ca	0.16±0.02	0.14±0.02	0.11±0.02	0.15±0.01
P	1.53±0.04	1.28±0.06	0.86±0.04	1.34±0.03
Na	0.78±0.05	0.67±0.04	0.43±0.06	0.59±0.02
K	0.72±0.02	0.64±0.05	0.47±0.03	0.52±0.07
Mg	0.07±0.06	0.06±0.04	0.05±0.02	0.06±0.03
Fe	0.06±0.02	0.06±0.02	0.05±0.03	0.06±0.03
Cu	0.06±0.01	0.06±0.01	0.04±0.02	0.05±0.02
Mn	0.05±0.01	0.05±0.01	0.03±0.02	0.06±0.03
Zn	0.05±0.02	0.05±0.02	0.04±0.03	0.06±0.03

Note: Different superscripts in the same row indicated significantly different (P<0.05).

Phytase enzyme at 500, 1000, and 1500 FTU/kg of feed increased digestibility coefficient of common carp protein significantly (P<0.05), compared to that of the samples given feed without phytase enzyme (Table 2). An increase in protein digestibility increases the digestibility of amino acids significantly (Table 6). Table (6) shows that alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, and valine increased significantly (P<0.05) in the experimental feeds containing phytase enzymes.

Table 6. Coefficient digestibility of amino acids (AA) of soy-based test feed as a source of vegetable protein given to common carp at the rearing stage, supplemented with different doses of phytase enzymes.

AA digestibility coefficients (%)	Feed test			
	A (0 FTU/kg feed)	B (500 FTU/kg feed)	C (1000 FTU/kg feed)	D (1500 FTU/kg feed)
Alanine	68.37[b]	70.42[a]	72.24[a]	71.86[a]
Arginine	74.52[b]	77.68[a]	77.90[a]	78.12[a]
Aspartic acid	73.28[b]	75.36[a]	76.05[a]	76.29[a]
Cysteine	74.63[b]	79.34[a]	80.68[a]	80.69[a]
Glutamic acid	84.45[b]	88.27[a]	88.53[a]	88.82[a]
Glycine	75.26[b]	80.64[a]	80.75[a]	80.88[a]
Histidine	80.86[b]	88.35[a]	88.67[a]	88.92[a]
Isoleucine	72.42[b]	78.48[a]	78.79[a]	78.96[a]
Leucine	71.25[b]	78.54[a]	78.70[a]	78.84[a]
Lysine	82.39[b]	87.68[a]	86.94[a]	86.97[a]
Methionine	75.10[b]	80.72[a]	81.10[a]	81.14[a]
Phenylalanine	73.48[b]	81.25[a]	81.36[a]	81.38[a]
Proline	74.53[b]	80.87[a]	80.92[a]	80.95[a]
Serine	76.37[b]	82.46[a]	82.68[a]	82.87[a]
Taurine	80.18[b]	83.56[a]	83.84[a]	83.84[a]
Threonine	76.23[b]	84.62[a]	84.62[a]	84.63[a]
Tryptophan	88.67[b]	90.49[a]	90.56[a]	90.56[a]
Tyrosine	78.18[b]	82.58[a]	83.12[a]	83.12[a]
Valine	76.75[b]	83.43[a]	83.43[a]	83.40[a]
Amino acid total	145.692[b]	81.79[a]	82.16[a]	82.22[a]
Average	76.68[b]	81.79[a]	82.16[a]	82.22[a]

Note: Different superscripts in the row indicated significant differences ($P<0.05$).

DISCUSSION

The addition of phytase enzyme to the soybean meal-based experimental feed affected significantly EFU, PER, FCR, RGR, and no significant ($P>0.05$) difference was detected in SR of common carp at the rearing stage. EFU, PER, FCR and RGR values of rearing stage common carp were higher after being fed with phytase enzymes (500, 1,000 and 1,500 FTU/kg feed). Adding phytase enzymes to feed can increase the hydrolysis of phytic acid so that the intestinal system can absorb nutrients bound by phytic acid and increase protein digestibility **(Hassaan *et al.*, 2013)**. **Cao *et al.* (2007)** stated that, the breakdown of phytic acid could increase the absorption of nutrients since the hydrolysis reaction by the phytase enzyme can reduce phytic acid and release protein and mineral bonds. **Wang *et al.* (2009)** postulated that, the breakdown of protein phytate complex bonds can increase the activity of trypsinogen and trypsin enzymes, breaking down

proteins into amino acids. The highest EFU, PER, FCR, and RGR values were observed in common carp after being given the experimental feed with 1000 FTU/kg phytase enzyme. The phytase enzyme at 1000 FTU/kg of feed hydrolyzed effectively the phytate complex compounds via releasing phosphorus, protein and minerals from soybean meal. The release of complex phytate compounds can facilitate the absorption of phosphorus, protein and minerals and increase growth **(Tahoun *et al*., 2009)**.

Table (3) shows that phytase enzyme supplementation in feed affects body protein and ash content but does not affect fat content or dry body matter of common carp grow-out stages. Common carp fed with phytase enzyme showed higher body protein content than samples fed without phytase enzyme. This may be due to the ability of the phytase enzyme to increase protein availability by hydrolyzing the phytate-protein complex in the intestine of fish and neutralizing the negative effect of phytate on protein **(Liebert & Portz, 2005)**. In this study, an increase in PER supported protein content in common carp bodies. Common carp fed with 1000 FTU/kg feed of phytase enzyme had the highest increase in protein digestibility, followed by the highest feed utilization efficiency (EFU), compared to other experimental feeds. Supplementation of phytase enzymes in feed increases protein digestibility in a direct proportion to the increased efficiency of feed utilization in fish **(Biswas *et al*., 2019)**. The effect of phytase enzyme on protein digestibility was previously reported by **Shapawi *et al*. (2013)** on *Ephinephelus fuscoguttatus*, **Bulbul *et al*. (2015)** on *Marsupenaeus japonicas* and **Hussain *et al.* (2017)** on *Labeo rohita*. **Bulbul *et al*. (2015)** stated that, adding phytase enzymes to feed could increase the bone ash concentration of *Marsupenaeus japonicas*' body. **Liebert and Portz (2005)** also reported that, the ash content of the scales and spine of *Oreochromis niloticus* increased when fed a diet containing the phytase enzyme. **Liebert and Portz (2005)** study coincides with the current findings, where common carp fed with phytase enzyme supplementation had a higher body ash content than without phytase enzyme supplementation.

In addition, the present study showed that common carp fed with supplementation of phytase enzymes increased the digestibility of minerals in the body compared to those without supplementation. A similar statement by **Sanz-Penella *et al*. (2012)** reported that fish-fed feed supplemented with phytase enzymes showed increased digestibility of minerals (Ca, Mg, Na, K, Fe, Cu, Zn, and Mn). Furthermore, the results of the study showed the highest mineral digestibility values of Ca, P, Na, K, Mg, Fe, Cu, Mn, and Zn in rearing stage common carp fed with 1000 FTU/kg phytase enzyme supplementation and the lowest in 0 FTU/kg feed. In general, the digestibility of minerals began to increase at a phytase enzyme dose of 500 FTU/kg and reached a maximum at a phytase supplementation dose of 1000 FTU/kg. It is evident from various studies that phytase supplementation has shown an effect up to a certain dose level on *Labeo rohita* **(Baruah *et al*., 2007)**, Atlantic salmon (*Salmo salar*) **(Carter & Sajjadi, 2011)** and seabream **Salem *et al.* (2022)** on *Sparus aurata*.

Phytase supplementation in plant-based diets reduces phosphorus excretion into the aquatic environment. Table (5) shows that minimum phosphorus minerals are excreted in the aquatic environment through feces compared to the samples fed feed without phytase enzyme supplementation. This study showed that higher phosphorus digestibility reduced phosphorus release in common carp fed with phytase enzyme supplementation, compared to those without supplementation. The supplementation of

phytase enzymes in feed has proven to be very beneficial in developing environmentally friendly grow-out feed for common carp by increasing mineral digestibility and reducing mineral excretion into the aquatic environment, and it is expected to help reduce water pollution **(Goda, 2007; Gabriel *et al.*, 2007; Hussain *et al.*, 2011b, Hussain *et al.*, 2014)**.

In this study, 1000 FTU/kg phytase enzyme was the best dose where the maximum utilization of this mineral occurs in the fish body, and the minimum minerals are excreted in the aquatic environment through feces. **Hussain *et al.* (2014)** attributed the reduced excretion of minerals in fish feces to the hydrolysis of phytate content by supplementing phytase enzymes to utilize more minerals. Furthermore, **Liu *et al.* (2013)** suggested that, phytase enzyme supplementation in feed at 1000 FTU/kg positively affected the utilization of P, Ca, Mg and Zn. In this study, the reason for the positive effect of phytase supplementation is that the phytase enzyme has hydrolyzed the phytate-mineral complex, resulting in the release of chelated minerals, leading to increased digestibility of these minerals **(Wang *et al.*, 2009; Hussain *et al.*, 2011b)**. Similar research results were reported by **Nwanna *et al.* (2007)**, who found that most of the action of phytase was focused on phytate degradation, resulting in the release of more minerals and ending in increased mineral digestibility. The supplementation of the phytase enzyme at 1000 FTU/kg was the best dose for mineral digestibility for common carp at the rearing stage. In contrast to the results of the present studies, the best dose of phytase enzyme was 500 FTU/kg for several fish species, including *Pangasius pangasius* **(Debnath *et al.*, 2005)**, *Labeo rohita* **(Baruah *et al.*, 2007)** and *Cyprinus carpio* L. **(Sardar *et al.*, 2007)**.

Cao *et al.* (2007) suggested that, many factors such as fish species, different sources of phytase, feed formulations, and parameters observed influential differences in fish requirements for supplementing phytase enzymes in the feed. Thus, there is no comparative study of phytase enzyme supplementation in feed for different fish species. The variation in the dose of phytase enzyme in feed depends on the ingredients and composition of the feed formulation and the species studied. Thus, studying the mechanism of phytic acid degradation in different fish species is necessary because it depends on different feed formulations, special characteristics of the digestive tract and varied activities of various sources of phytase enzymes.

Phytic acid in feed can bind non-selectively to amino acids and reduce the availability of amino acids **(Kumar *et al.*, 2012)**. In addition, phytic acid has also been shown to inhibit the activity of enzymes, including trypsin and pepsin **(Morales *et al.*, 2014)**. Supplementation of phytase enzymes in feed can increase the availability of protein and amino acids through breaking down phytic-protein complexes and neutralizing the negative effects of phytate on protein and other nutrients in feed **(Hussain *et al.*, 2011b)**. **Hussain *et al.* (2011b)** revealed that, 500 and 2,000 IU/kg phytase increased the protein digestibility coefficient significantly. Increased protein

digestibility means increased amino acid digestibility. The apparent digestibility coefficients (Table 7) of alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine and valine increased significantly with feed containing phytase enzyme supplements of 500, 1000 and 1,500 FTU/kg feed.

CONCLUSION

The supplementation of phytase enzymes in feed with soybean meal had a significant effect ($P<0.05$) on growth, mineral digestibility, amino acid digestibility and body chemical composition of common carp grow-out stages. Growth, mineral digestibility, amino acid digestibility and body chemical composition of common carp, especially protein and ash content, significantly increased after phytase enzyme supplementation, compared to those without supplementation. The phytase enzyme at 1000 FTU/kg feed was the best dose for the rearing stage of the common carp.

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