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RESEARCH ARTICLE

EFFECT OF PHYTASE SUPPLEMENTATION ON *LABEO ROHITA* FINGERLINGS THAT ARE FED BY PHYTASE SUPPLEMENTED DDGS BASED DIET

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ABSTRACT

Phytate is not hydrolyzed by fish because natural phytase activity not present in some agastric fishes, phytate is present in grain products as the main form of phosphorus. The present study was aimed to check the effect of phytase supplementation on muscle proximate composition in *Labeo rohita* fingerlings that are fed by phytase supplemented distiller's dried grain with soluble. Six experimental diets were prepared by supplementing phytase at graded levels. D1 diet contains no supplemented phytase, while, D2, D3, D4, D5 contain 250, 500, 750, 1000, and 1250 FTU/kg phytase, respectively. The experiment continues for eight weeks. Water quality parameters including pH, temperature, and dissolved oxygen were controlled throughout the experiment. Results were analyzed through the Student-Newman-Keuls test for significant differences. Supplementation of phytase decreased the moisture and fat content of muscles in L. rohita fingerlings. Supplementation of phytase increases the crude protein and crude ash content of muscles in L. rohita fingerlings. In conclusion, phytase showed randomized responses to muscle composition.

KEYWORDS

Phytase, Labeo rohita, Distiller's dried grain with soluble, Phytase supplementation, Rohita fingerlings feeding, Phytase supplemented, DDGS based diet.

1. INTRODUCTION

The aquaculture sector is developing more efficiently than other food-producing industries (Yildirim*et al*., 2014). However, the development of this industry is limited due to economic factors such as feed's cost (Yildirim*et al*., 2014). However, fish meal prices are tremendously increasing in the last few years, due to over-exploitation of natural fishing grounds, the availability of fish meal has been alarmingly decreased (Lech and Reigh, 2012).

To improve the production of fish the most important feed is supplemental feed. In semi-intensive culture, artificial feed plays a major role in which a greater number of fish is needed whose natural fecundity of the water can tolerate. The entire demand for fish nutrition relies on the food that's why the importance and function of supplemented feed in intensive fish culture could not be neglected. (Malik et al., 2020). A by-product of the ethanol distillery industry is Distiller's dried grains with solubles (DDGS), is less expensive on a per-unit protein basis than soybean meal. Thus, for aquaculture business, DDGS is an economical alternative of fishmeal and other plant-based proteins.

DDGS based diets were compared with a fish meal-based control diet. Fish fed 20% DDGS accumulated more body fat as compared to other to diets (control and 10% DDGS). No significant difference was observed in proximate (crude lipid, moisture contents, crude protein, and ash) composition. Similarly, no significant difference was observed in the protein digestibility coefficient. It was concluded that DDGS even supplemented with amino acids or phytase will lead to decreased weight in rainbow trout at dietary concentrations of at 10% or greater (Barnes *et al*., 2012).

Researchers are motivated to identify economical alternatives to fishmeal due to its increasing price (Shapawi *et al*., 2013). As DDGS content of phosphorus is low, replacement of soybean meal with DDGS will reduce the phosphorus level in the diet (Liener, 1994). It reduces the discharge of phosphorus and other minerals from fish farms due to which environmental pollution is also reduced. Plant by-products that we use in fish feeds have anti-nutritional factors like phytic acid and phytate (Baruah *et al*., 2004). Phytate is not accessible for monogastric and agastric fishes is about 80% of total plant phosphorus content (NRC, 1993), which hurt the digestive ability of fish. Growth performance of fish is affected due to shortage of many elements like positively charged mineral cations, proteins, positively charged amino acids, and fatty acids as negatively charged phytate chelates with them and they become unavailable to fish (Usmani and Jafri, 2002)

Growth performance of agastric fishes such as *Labeo rohita* will be reduced if they are fed phytate rich plant ingredients, as these fishes have no natural phytase activity (Baruah *et al.,* 2004; Cao *et al*., 2007; Hussain *et al*., 2011a). Utilization and bio-availability of plant phosphorus by fish can be improved by the addition of phytase in the feed of fish. (Vielma *et al.,* 1998; Baruah *et al*., 2007). Microorganisms produce phytase enzymes it also found in some plant ingredients (Cao *et al*., 2007). Phytate acid can be reduced by adding this enzyme in the diet (NRC, 1993). Phytase

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improves absorption, regulation, and excretion of nutrients and hydrolyzes phytate acid to become inositol and phosphate acid (Chung, 2001). In Pakistan *Labeorohita* commonly known as rohu is the main major carp species (Abid and Ahmed, 2009; Hussain *et al.*, 2011a). Due to disease resistance capacity, unique flavor, and rapid growth, the demand of *L. rohita* is dominant. The focus of this study is to determine the effect of phytase supplementation on muscle proximate composition in *Labeo rohita* fingerlings fed phytase supplemented distiller's dried grain with a soluble based diet.

2. Materials and Methods

A study conducted in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

2.1 Methodology

Labeorohita fingerlings were brought from Government Fish Seed Hatchery, Faisalabad. They acclimatized in v-shaped (UA system) tanks, specially designed to collect feces (figure 1), for 15 days. Each tank has eighteen fishes. The basal diet (Table 1) was given to fingerlings once in a day (Allan and Rowland, 1992) on apparent satiation. Jenway pH meter model 3510 and D.O. meter model 970 were used to check water quality variables like pH, and dissolved oxygen. These parameters were controlled between a specific range; temp 24.9-28.7°C, DO 5.8-7.3 mg/L, and pH 7.4-8.6. The capillary system was used to give proper oxygen to every tank.

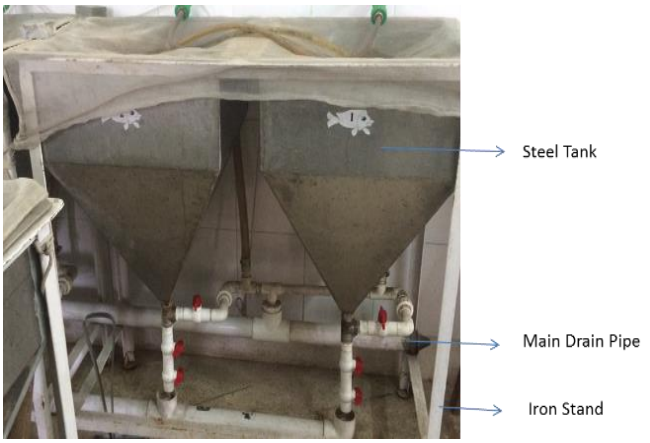

Figure 1: Modified UA system showing the steel tank and main pipe

2.1.1 Feed ingredients and experimental diet:

From the local poultry feed market, the feed ingredients were bought and before preparing the experimental diet it was subjected to chemical composition following (AOAC, 1995). Before adding in the experimental diet feed ingredients ground and sieved to require particle size (Table 1).

Table 1: Composition (%) of the experimental diet

Ingredient	Percentage
Fishmeal	15
Corn DDGS	53
Soybean meal	15
Wheat flour	7
Fish oil	6
Vitamin C	1
Vitamin premix*	1
Mineral mixture**	1
Choline chloride	0.5
Chromic oxide	0.5
Total	100

*Each Kg of Vitamin premix contains

Vitamin A 15 M.I.U.	Vitamin D3 3 M.I.U.	Nicotinic acid 25000mg
Vitamin B1 5000 mg	Vitamin E 6000 IU	Vitamin B2 6000 mg
Vitamin K3 4000 mg	Vitamin B6 4000 mg	Folic acid 750 mg
Vitamin B12 9000 mcg	Vitamin C 15000mg	Calcium pantothenate 10000mg

**Each Kg mineral granules contains

Ca (Calcium) 155gm	Mn (Manganese) 2000mg	P (Phosphorous) 135gm
Cu (Copper) 600mg	Mg (Magnesium) 55gm	Co (Cobalt) 40mg
Fe (Iron) 1000 mg	I (Iodine) 40mg	Zn (Zinc) 3000 mg
Se (Selenium) 3mg	Na (Sodium) 45gm	

Six diets were prepared by adding phytase at levels of 0, 250, 500, 750, 1000 and 1250 FTU/kg and labeled as D1, D2, D3, D4, D5, and D6.

Table 2: Chemical composition (%) of feed (Dry Basis)

Diet	Phytase (FTU/kg)	Dry matter (%)	Crude protein (%)	Crude fat (%)
D1	0	91.905	30.849	8.7845
D2	250	91.565	30.9965	9.253
D3	500	92.29	31.264	9.1875
D4	750	92.71	30.701	9.112
D5	1000	91.64	31.8945	9.7445
D6	1250	92.225	31.194	8.9235

2.1.2 Formation of experimental diet:

For 10-20 minutes, all dry diet ingredients were blended in an electric mixer and fish oil was added moderately with constant stirring. As an inert marker, chromic oxide (0.5%) was added. Phytase was added at the levels of 0, 250, 500, 750, 1000, and 1250 FTU/kg to dry mixed ingredients to make six test diets. To make suitable dough of diet add ten to fifteen percent water in it and mix slowly and floating pellets were made by using lab extruder.

2.1.3 Feeding Protocol and Sample Collection

Experimental diets were given to *Labeorohita* fingerlings at a rate of 2% of their wet weight. Replicates were assigned to each test diet. By opening the valves of each tank the uneaten diet was drained out after three hours of feeding. To remove particles of diets from tanks were washed completely and refilled. Again put the fish in the tank. After two hours by opening the valve I and II of the collection tube, the feces were collected. Feces were collected carefully so that fecal string did not break because it minimizes the nutrient leaching. Then feces were dried in an oven at 60°C then crushed and lead to chemical analysis. The collection was continued until 5 g of fecal matter was collected.

2.2 Chemical analysis

In pestle and mortar, the diet sample and fish muscle sample were homogenized and subjected to standard methods (AOAC, 1995). The sample was dried in an oven at 105°C for 12 hours to find out the moisture content. Kjeldahl apparatus was used for crude protein, and soxhtecHT2 1045 system was used for crude fat extraction (Bligh and Dyer, 1995). By igniting samples in an electric furnace (Eyela-TMF 3100) at 605°C for 12 hours crude ash was determined.

2.2.1 Dry matter

Fill the Petri dish by taking 1 g of a sample of feed and fish muscles and

located in the oven for 12 hours at 105°C. Then for 5 minutes place it in desiccators and weighed. For taking constant weight again put it in the oven for 1-2 hours. Moisture was determined by the loss in weight and dry matter percentage was calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{W1-W2} \times 100}{\text{Weight of sample}}$$

Dry matter = 100 – moisture %

Where,

W_1= weight of Petri dish + sample before drying

W_2= weight of Petri dish + sample after drying

2.2.2 Crude protein

Micro Kjeldahl apparatus was used to estimate Crude protein from feed and fish muscle samples. A digestion mixture was prepared by mixing of K_2SO_4, CuSO4, and $FeSO_4$in the proportion of 90:7:3. Take 5 g of digestion mixture, 30 ml of H_2SO_4, and 1 g of dried sample in Kjeldahl flask. Then the flask was kept on hot plate and heat until a transparent pure greenish solution is obtained. Cooldown the digestion material and make it diluted by the addition of 250ml of distilled water. For distillation take 10 ml of digestion mixture and 10 ml of 40% NaOH and distill with steam. Take 10 ml 2% Boric acid in a beaker and add 2 drops of methyl orange indicator and place for ammonia collection. Ammonia collected for 2 minutes after changing the color of the indicator from pink to golden yellow. Then titrate this solution against 0.1 N H_2SO_4. The percentage of N_2 in the sample was calculated by the following formula.

$$\text{Nitrogen \%} = \frac{\text{Volume of } H_2SO_4 \text{used} \times \text{Normality of } H_2SO_4 \times 0.014 \times 250}{\text{Weight of sample} \times 10} \times 100$$

Where,

0.014= Standard volume of 0.1 N H_2SO_4 used to neutralize 1 ml of ammonia

250= Dilution of the digestion mixture

100= for a percentage of N_2

10= Volume of digestion and diluted sample used

Crude protein in the sample was calculated by the following formula

Crude protein (%) = N_2 x 6.25

2.2.3 Crude fat extraction

Soxtec system was used for crude fat determination using petroleum ether. In Soxtec thimble, 1 g of a sample is placed and attached to an adapter. At top of thimble cotton wool is placed. The thimble was inserted into the condenser. In the weighted extraction, the cup adds 50-70 ml ether and weigh it. Turn on the main switch for electricity and open water tap. The extraction cup was held strongly into the condenser. The thimble dipped in the solvent. Make sure that condenser valves were untied. The regulators of condensers were kept close during rinsing. Valves of condensers were opened and removed from the extraction cup. For drying the extraction cup with less quantity of solvent, fat or petroleum ether was located in the oven. Extraction cup put in desiccators for 5 minutes after drying and weighing. Following formula used to calculate the sample of crude fat.

$$\text{Fat (\%)} = \frac{W3 - W2}{W1}$$

Where

W_1= Weight of sample (g)

W_2= Weight of empty extraction cup (g)

W_3= Weight of extraction cup + residue weight (g)

2.2.4 Statistical analysis

Following Steel *et al.*, (1996) the data of muscle proximate were subjected to a one-way analysis of variance. By Tukey's Honestly Significant Difference Test the differences among means were compared and considered significant at P<0.05 (Snedecor and Conhran, 1991). This Version 6.303 of Costate Computer Software was used for statistical analysis.

3. RESULTS

3.1 Moisture content (%) in the muscles of *Labeorohita* fingerlings

The effect of graded levels of phytase supplementation on the moisture contents in the muscles of *Labeorohita* fingerlings is given in table 3.1. One-way analysis of variance (ANOVA) showing the effect of supplementation of dietary phytase is given in table 3.2. Fig 3.1 shows the moisture content (%) in the muscles of *Labeorohita* fingerlings. The resulting data (P<0.05) showed that the supplementation of phytase decrease the moisture contents in the muscles of the *Labeorohita* fingerlings. The maximum decrease in moisture was observed in diet D3 containing phytase level 500 FTU/kg. While the minimum decrease in moisture content was recorded in the muscles of the *Labeorohita* fingerlings in diet D2 containing phytase level 250 FTU/Kg.

Table 3.1: Effect of graded level of phytase supplementation on moisture content (%) in muscles of Labeorohita fingerlings

Diet	Phytase level (FTU/Kg)	Moisture (%)
D1	0	76.28[a]
D2	250	76.56[a]
D3	500	75.91[a]
D4	750	75.93[a]
D5	1000	76.09[a]
D6	1250	75.98[a]
PSE		0.3277702702
ANOVA		**P-value**
Phytase		0.7072 ns

Table 3.2: One-way analysis of variance (ANOVA) showing the effect of graded levels of phytase supplementation on moisture content (%) in muscles of *Labeorohita* fingerlings

Sources	DF	SS	MS	F	P
Main effects					
Phytase	5	0.6395	0.1279	0.5952529	0.7072 ns
Error	6	1.2892	0.2148667		
Total	11	1.9287			

Results were considered significant at p<0.05

Data are means of two replicates

PSE= pooled SE= $\sqrt{MSE/n}$ (where MSE= mean squared error, n=number of replicates)

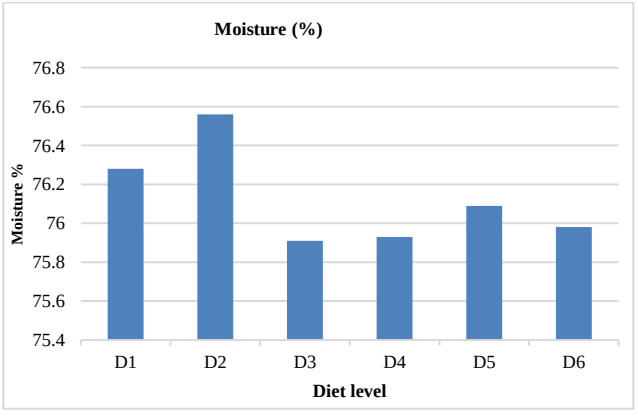

Figure 2: Graphical illustration of the effect of graded level of phytase supplementation on moisture content (%) in muscles of *Labeorohita* fingerlings

3.2 Crude protein (%) in the muscles of *Labeorohita* fingerlings

The effect of graded level of phytase supplementation on the crude protein in the muscles of *Labeorohita* fingerlings is given in table 3.3. One way analysis of variance (ANOVA) showing the effect of supplementation of dietary phytase is given in table 3.4. Fig 3.2 shows the protein (%) in the muscles of *Labeorohita* fingerlings. The resulting data (P<0.05) showed that the supplementation of phytase increase the crude protein in the muscles of the *Labeorohita* fingerlings. The maximum increase in crude protein was observed in diet D3 containing phytase level 500 FTU/Kg. While the minimum increase in crude protein was recorded in the muscles of the *Labeorohita* fingerlings in diet D2 containing phytase level 250 FTU/Kg.

Table 3.3: Effect of graded level of phytase supplementation on crude protein (%) in muscles of *Labeorohita* fingerlings

Diet	Phytase level (FTU/Kg)	CP (%)
D1	0	17.71[d]
D2	250	17.97[c]
D3	500	18.4[a]
D4	750	18.24[b]
D5	1000	18.06[c]
D6	1250	18.06[c]
PSE		0.0292976108
ANOVA		**P-value**
Phytase		0.0000***

Results were considered significant at p<0.05. Data are means of two replicates. PSE= pooled SE= $\sqrt{MSE/n}$ (where MSE= mean squared error, n=number of replicates)

Table 3.4: One-way analysis of variance (ANOVA) showing the effect of graded levels of phytase supplementation on crude protein (%) in muscles of *Labeorohita* fingerlings

Sources	DF	SS	MS	F	P
Main effects					
Phytase	5	0.555866	0.1111733	64.761165	0.0000***
Error	6	0.0103	0.0017167		
Total	11	0.56895			

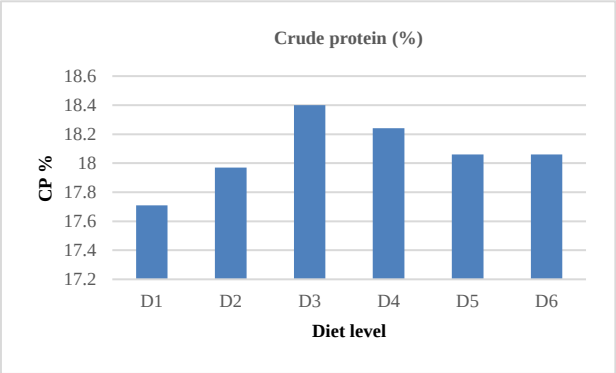

Figure 3.2: Graphical illustration of the effect of graded level of phytase supplementation on crude protein (%) in muscles of *Labeorohita* fingerlings

3.3 Crude fat (%) in the muscles of *Labeorohita* fingerlings

The effect of graded level of phytase supplementation on the crude fat in the muscles of *Labeorohita* fingerlings is given in table 3.5. One-way analysis of variance (ANOVA) showing the effect of supplementation of dietary phytase is given in table 3.6. Fig 3.3 shows the crude fat (%) in muscles of *Labeorohita* fingerlings. The resulting data (P<0.05) showed that the supplementation of phytase decrease the crude fat in the muscles of the *Labeorohita* fingerlings. The maximum decrease in crude fat was observed in diet D4 containing phytase level 750 FTU/Kg. While the minimum decrease in crude fat was recorded in the muscles of the *Labeorohita* fingerlings in diet D6 containing phytase level 1250 FTU/Kg.

Table 3.5: Effect of graded levels of phytase supplementation on crude fat (%) in muscles of *Labeorohita* fingerlings

Diet	Phytase level (FTU/Kg)	CF (%)
D1	0	4.24[a]
D2	250	3.4[d]
D3	500	3.4[d]
D4	750	3.31[d]
D5	1000	3.82[c]
D6	1250	4.07[b]
PSE		0.0449071264
ANOVA		**P-value**
Phytase		0.0000***

Results were considered significant at p<0.05

Data are means of two replicates

PSE= pooled SE= $\sqrt{MSE/n}$ (where MSE= mean squared error, n= number of replicates)

Table 3.6: One-way analysis of variance (ANOVA) showing the effect of graded levels of phytase supplementation on crude fat (%) in muscles of *Labeorohita* fingerlings

Source	DF	SS	MS	F	P
Main Effects					
Phytase	5	1.554066667	0.3108133	77.061157	.0000***
Error	6	0.0242	0.0040333		
Total	11	1.578266667			

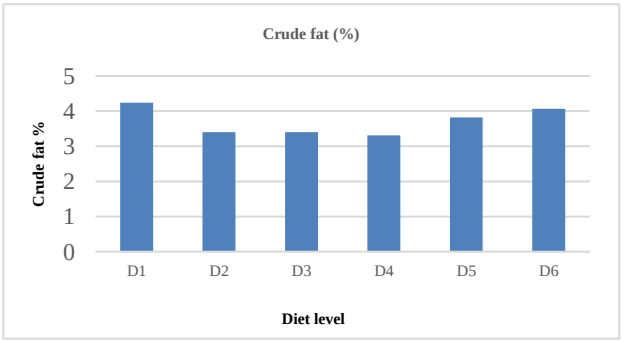

Figure 3.3: Graphical illustration of the effect of graded level of phytase supplementation on crude fat (%) in muscles of *Labeorohita* fingerlings

4. Discussion

Aquaculture is developing more than any other industry in Pakistan. The availability of food for fish is the major problem of fish farmers due to the expensive rate of fish meal which used as a protein source in fish feed. The alternative of fish meal is planted by-product which also has high protein energy. Distiller's dried grain with soluble is also a plant by-product that can be used in place of fish meal. However, DDGS have low phosphate rate in it and therefore discharge low phosphate in fish farms and reduce the environmental pollution. These plants by-product have anti-nutritional factor-like phytic acid and phytate. Monogastric and agastric fishes cannot digest this phytate and it hurts the digestibility of fish. Phytate acid can be reduced by adding phytase enzymes in the diet. Phytase improves absorption, regulation, and excretion of nutrients and hydrolyzes phytate acid to become inositol and phosphate acid. The basic purpose of our study is to make a cheap and environment pleasant fish diet that is easily available to fish farmers. We experiment on *Labeorohita* fingerlings which are fed on phytase supplemented DDGS based diet. We prepared six diets

by adding phytase at levels of 0, 250, 500, 750, 1000 and 1250 FTU/kg and labeled as D1, D2, D3, D4, D5, and D6. Fingerlings were fed according to protocol. The results of muscle proximate analysis showed that phytase decreases the crude fat (%) and moisture content (%). While it increases the crude protein (%) and crude ash (%) in muscles of *Labeorohita* fingerlings.

A similar experiment was done by Tahoun et al (2009) on fingerlings of Nile tilapia (*Oreochromis niloticus*). Five experimental diets with graded levels (0, 75, 150, 225, 300 mg/kg) were formulated and applied to randomly distributed fish in hapas (36 fish/hapas). The feeding trial lasts for 82 days. Finally, it was concluded that growth performance and feed utilization is significantly improved in Nile tilapia fingerlings fed on DDGS based diet supplemented up to 150 mg/kg phytase. Cheng and Hardy (2004) also conducted two experiments to investigate the effect of microbial phytase supplementation in corn distiller's grain with soluble (DDGS) on growth performance and apparent digestibility of rainbow trout (*Oncorhynchus mykiss*). In experiment 1 the phytase was supplemented at the levels of 0, 300, 600, 900, and 1200 FTU/kg of diet. Proximate analysis of fish indicated that the fish fed with DDGS diet supplemented with phytase were: gross energy, 50.5-66.6%; dry matter, 49.1-58.6%; minerals, 7.3-99.7%; amino acids: 73.9 to 96.8%; crude protein, 80.0-91.9% and crude fat, 78.9-88.9%. In2nd experiment 15%DDGS based diet supplemented with methionine and lysine was used to evaluate mineral supplemental levels in rainbow trout. Six diets with trace mineral supplementation at 0.02%, 0.04%, 0.06%, 0.08% and 0.1% were formulated. Phytase was not supplemented in a control diet while supplemented at 500 FTU/kg in all other experimental diets. After 10 weeks, it was concluded that the phytase supplementation proves to be effective for releasing most trace minerals and could reduce the supplementation of trace mineral in the diet of rainbow trout.

Hung *et al*. (2015) prepared six diets with different levels of Di-calcium phosphate (0, 5, 10 and 15 g/kg) and fungal phytase (750 and 1500 FTU/kg) having similar content of nitrogen (320g/kg) and lipids (60g/kg). Feeding trial lasts for 3 months in which tra catfish (*Pangasianodon hypophthalmus*) were randomly assigned to the experimental diets. At the termination of the feeding trial, considerably higher values of protein efficiency ratio, growth performance, and phosphorus retention were shown by fish fed the diets supplemented with 750 and 1500 FTU/kg phytase and 15g/kg DCP as compared to those fed the control diet (p<0.05). A significant increase was observed in whole-body ash and phosphorus concentration of fish fed with 10g/kg and 15g/kg DCP diets than those fed on the control diet. Fish fed on phytase supplemented diets showed a higher value of apparent digestibility coefficient of phosphorus. Results suggested that growth performance, phosphorus, and feed utilization values were enhanced by phytase supplementation at 750FTU/kg and 1500 FTU/kg. Infeed of tra catfish, DCP, or other phosphorus sources can be replaced by phytase supplementation and thus phosphorus excretion is controlled, reducing the aquatic pollution. Another experiment was done by Hussain *et al*. (2015) to evaluate the effect of phytase supplementation on mineral digestibility and growth performance in fingerlings of *Labeorohita*. Seven experimental diets were formulated by adding different levels of phytase (0, 250, 500, 750, 1000, 1250, and 1500 FTU/kg) to the basal diet. Fish fed on T_4 with 750 FTU/kg phytase supplementation exhibited the highest performance. Finally, resulted in data leads to conclude that 750 FTU/kg phytase level diet was proved to be more effective for releasing the chelated minerals, hence increase the growth performance of *L.rohita* fingerlings. It was concluded that phytase supplementation in plant-based material could help reduce the mineral excretion in aqua bodies and hence reduced aquatic pollution.

5. Conclusion

We use plant by-products in fish feed as a source of protein however, it also contains phytate as part of phosphorus. Phytate is not hydrolyzed by fish because natural phytase activity not present in some agastric fishes. Therefore it is necessary to supply phytase enzyme artificially as natural phytase activity not present in some agastric fishes. This study aims to check the effect of phytase supplementation on muscle proximate composition in *Labeo rohita* fingerlings that are fed with phytase supplemented distiller's dried grain with soluble based diet. Based on the results of existing ponders, it can be concluded that phytase has a randomized effect on muscle composition of *Labeorohita* fingerlings. Results were analyzed through the Student-Newman-Keuls test for significant differences. Supplementation of phytase significantly (p<0.05) decreased the moisture and fat content of muscles in *L. rohita* fingerlings and significantly (p<0.05) increases the crude protein and crude ash content of muscles in *L. rohita* fingerlings. In conclusion, phytase supplementation may improve the meat quality of *Labeorohita* fingerlings

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