Short Communication

Effect of Phytase Supplemented Diet on Whole Body Proximate Composition of *Labeo rohita* Fingerlings

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**ABSTRACT**

Phytate, bound form of phosphorus and some other minerals, is present in plant products and it cannot be consumed by fish because there is no phytase activity in agestric fish. This study was performed to determine the impact on proximate composition of whole-body of *Labeo rohita* (fingerlings) fed distiller’s dried grains with solubles based diet, supplemented with phytase. Six different feeds were formulated by supplementing different concentrations of phytase, i.e. D1, without phytase supplementation, D2, D3, D4, D5, and D6 with 250, 500, 750, 1000 and 1250 FTU phytase per kilogram of diet, respectively. An experiment was conducted for 2 months. During this experiment, water quality parameters were also examined e.g. pH, Dissolved oxygen and temprature. Results were examined by Tukey’s Honestly Significant Difference Test. Phytase supplementation decreased the moisture content and increased the crude ash, protein and fat significantly in proximate composition of whole-body of the fish and hence, improved the meat quality of *Labeo rohita* fingerlings.

Aquaculture includes the rearing or culturing of aquatic organisms such as fish, crustaceans and molluscs. For most of the aquatic species, a major portion (60%) of the farm production cost usually goes to aquaculture diet (Cheng and Hardy, 2004). Considering the fish, aquaculture proves to be one of the most rapidly developing food industries in the world (Beveridge, 1984; Suguira et al., 1999). The main objective of aquaculture is to enhance the production of some required fish species to have maximum economic benefits (Goldburg and Triplett, 1997).

In aquafeeds, fishmeal is considered as one of the most important protein sources because of its balanced amino acid content and some important growth factors absent in plant based protein sources (Kousoulaki et al., 2009). Energy, minerals and essential fatty acids are also abundant in it (Webster et al., 1995). Anyhow, increasing requirement of fish meal leads to its reduced supply and high cost. So, to overcome these problems, other protein sources are explored for aquaculture (NRC, 1981). Thus, cost effective proteins are obtained from the plant source and considered to be the best alternate to fish meal (Gaylord et al., 2006).

Many sources of proteins are used for aquaculture but Distiller’s dried grain with solubles (DDGS) is considered as a cost effective source of protein for aquaculture diet comparative to fish meal (Bothast and Schlicher, 2005).

It is collected as a by-product in the production of ethanol during the process of fermentation (Coyle et al., 2004; Wu et al., 1994).

DDGS contain lower phosphorus content as compared to animal based proteins i.e fish meal (Cheng and Hardy, 2004). Wheat distiller’s dried grain (WDG) is readily available, cost effective and poor in anti-nutritional factors, thus it can be used as a substitute in the aquaculture feed industry as a source of protein (Randall and Drew, 2010; Reveco, 2010). Supplementation of DDGS in the diet resulted in the maintenance of fish growth along with reduced feed cost.

Phytate, an anti-nutritional factor, is present in DDGS containing approximately 80% phosphorus bound with other minerals. Chemically, it is considered the free state of inositol hexa-kisphosphate and a polyanionic compound having six groups of phosphate that chelates with positive ions e.g., magnesium, iron, calcium, copper, potassium and zinc forming insoluble salts. Thus, digestion of the minerals in the fish are severely affected (NRC, 1993; Papaytrophyn et al., 1999).

This problem is resolved by supplementing phytase in fish diet formulations. It belongs to a group of enzymes called myo-inositol hexa-phosphate phosphohydrolase. Phytate is added to fish diet to hydrolyze bound minerals in phytate, found in plant source diets, thus increasing the availability of nutrients to fish. Chelation of several cations with phytate is also impeded by this (Ravindran et al., 1995). Similarly, plants based protein digestibility is...
also enhanced with supplementation of phytase (Baruah et al., 2007a). Addition of phytase in aqua feeds also leads to
to reduction in water pollution by retaining the phosphorus in
fish body and thus reducing its excretion in feces (Baruah et al., 2004).

*Labeo rohita* is considered an important Indian
major carp that contributes almost eighty-seven percent of
total freshwater aquaculture (ICLARM, 2001). It is
heterosexual and usually cultured in a polyculture system.
It has become very important in aquaculture because of
its high commercial value (Jhingran, 1991). It can survive
well in freshwaters with an altitude less than 549. Adult
rohu is considered as both bottom and column feeders (Motwani and Bose, 1957). The main purpose of this
study was to find out the impact of phytase on proximate composition of whole-body of *Labeo rohita* (fingerlings)
fed phytase supplemented DDGS based diet.

**Materials and methods**

*Labeo rohita* (fingerlings) with average weight 3.25
g were brought from the Fish Seed Hatchery, Faisalabad.
Acclimatization was done for 14 days in new trial conditions
until trial has been started that lasted for 2 months. Trial fish
were kept in tanks (UA system) of 70L capacity. 108 fish
(18 fish/tank) were distributed in all treatments. Basal diet
(Table I) was given to fingerlings once a day throughout
the study period (Allan and Rowland, 1992). During
this study period, different parameters of water quality,
especially temperature, pH and dissolved oxygen were examined. pH was controlled between 7.4-8.6 and DO
temperature was controlled at 6.2 mg/L and 26.7°C. A
water pipeline system was used to provide proper aeration
to all treatments during the feeding trial.

For feed ingredients and experimental feeds dietary
components were obtained from the local fish feed mar-
ket and their chemical composition examined according to
(AOAC, 2000), before the experimental diet was prepared.
Before incorporating in the test diet (Table II) these feed
materials were crushed and sieved until required particle
size was attained.

In electric mixer, dried feed components were blended
for 10-20 min, with gradual addition of fish oil during
constant mixing. 0.5% chromic oxide, an inert marker, was
also added. Six experimental diets were formulated namely
D1, D2, D3, D4, D5 and D6 by supplementation of graded
levels of phytase to these dried, mixed food materials at 0,
250, 500, 750, 1000 and 1250 FTU/Kg of diet, respectively.
Dough of each test diet was prepared by adding 10-
15% water and then pellets were formed by extruder.

Experimental diets were given to fingerlings by two
percent of their live wet body weight. Feeding plates were
adjusted in the tanks to avoid the removal of feed particles
through sieve plates. Then 18 fish were stocked in each
tank and all experimental diets with their replicates were
allotted to all these treatments randomly. They were fed
for three h then valves of each tank were opened to drain
out the remaining diet. Diet particles were completely
removed by washing tanks, then water was filled again in
all the tanks and fish were restocked.

### Table I. Composition (%) of experimental diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15</td>
</tr>
<tr>
<td>Corn DDGS</td>
<td>53</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>15</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1</td>
</tr>
<tr>
<td>Fish oil</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mixture**</td>
<td>1</td>
</tr>
<tr>
<td>Chromic oxide</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

*Vitamin premix per Kg contains: Vitamin (A), 15 M.I.U; Vitamin (B6), 4000 mg; Vitamin (B12), 9000 mg; Vitamin (D3), 3 M.I.U.; Vitamin (K), 4000 mg; Nicotinic acid, 25000 mg; Vitamin (E), 6000 IU; Vitamin (B5), 6000 mg; Vitamin (B1), 5000 mg; Folic acid, 750 mg; Vitamin (C), 15000mg; Calcium pantothenate, 10000mg. **Mineral granules per Kg contains: Copper, 600mg; Selenium, 3mg; Magnesium, 55gm; Calcium,155gm; Iodine, 40mg; Zinc, 3000 mg; Phosphorous,135gm; Cobalt, 40mg; Iron, 1000 mg; Sodium, 45gm.

### Table II. Chemical composition (%) of feed (Dry basis).

<table>
<thead>
<tr>
<th>Diets</th>
<th>Phytase (FTU/kg)</th>
<th>Dry matter (%)</th>
<th>Crude protein (%)</th>
<th>Crude fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0</td>
<td>91.835</td>
<td>33.335</td>
<td>9.575</td>
</tr>
<tr>
<td>D2</td>
<td>250</td>
<td>92.15</td>
<td>32.93</td>
<td>10.355</td>
</tr>
<tr>
<td>D3</td>
<td>500</td>
<td>92.275</td>
<td>32.8</td>
<td>10.15</td>
</tr>
<tr>
<td>D4</td>
<td>750</td>
<td>92.08</td>
<td>32.76</td>
<td>10.31</td>
</tr>
<tr>
<td>D5</td>
<td>1000</td>
<td>92.065</td>
<td>33.105</td>
<td>10</td>
</tr>
<tr>
<td>D6</td>
<td>1250</td>
<td>92.025</td>
<td>32.945</td>
<td>9.65</td>
</tr>
</tbody>
</table>

Test diets and whole body samples were blended (by
mortar and pestle) and then examined through standard
Protocols (AOAC, 2000). To determine the dry matter, 1g
sample was oven dried for 12h at 105°C and transferred
to desicator for 5 min, then final weight of sample is
recorded. This process is repeated till the required weight
is obtained. Weight loss was considered as the moisture
content. Crude ash was determined by igniting sample at
65°C keeping it in electric furnace for 12 h to attain constant
Effect of Phytase Supplementation on Whole Body Proximate Composition of *L. rohita*

Weight. Crude fat analysis was performed through ether extraction (Bligh and Dyer, 1995) using soxhlet system and crude protein analysis through micro kjeldahl method. Dry matter, crude ash, fat and protein are then determined through following formulas:

\[
\text{Dry matter} = 100 - \text{Moisture contents } \%
\]

\[
\text{Moisture } (\%) = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100
\]

Where, \(W_1\) is weight of sample before drying+ Petri dish, and \(W_2\) is weight of sample after drying+ Petri dish.

\[
\text{Crude ash } (\%) = \frac{\text{Weight of ash(g)}}{\text{Weight of sample(g)}} \times 100
\]

\[
\text{Crude Fat } (\%) = \frac{W_3 - W_2}{W_1} \times 100
\]

Where, \(W_1\) is weight of sample used (g), \(W_2\) is weight of blank extraction cup (g), and \(W_3\) is weight of residue + extraction cup (g).

\[
\text{% of Crude protein contents } = \frac{\text{Nitrogen } (\%) \times 6.25}{\text{Weight of sample } \times 10}
\]

Where, 0.014 is standard volume of 0.1 N H\textsubscript{2}SO\textsubscript{4}, which is used to neutralize 1 ml of NH\textsubscript{3}, 250 is digestion mixture dilution, 100 is N\textsubscript{2} percentage, and 10 is volume of diluted and digested sample.

One-way analysis of variance was applied on resulted data of whole body proximate (Steel et al., 1996). For comparison of means, Tukey’s Honestly Significant Difference Test was performed and \(P<0.05\) was considered significant (Snedecor and Conhran, 1991).

**Results and discussion**

In current study, we used DDGS as fish diet because DDGS has more protein and fat content and less anti-nutritional substances (phytate) found in most of the other plant protein sources. Thus phytase is supplemented to DDGS for the digestibility of phytate. Phytases are actually a group of enzymes, which are considered very important to hydrolyze phytate in plants (Vohra and Satyanarayana, 2003).

Addition of different levels of phytase affects the % of moisture contents, in whole-body proximate of *Labeo rohita* (Table III). Results (\(P<0.05\)) showed that phytase supplementation reduces the moisture in fingerling body of the *L. rohita* which implies that dry matter has increased in fish as compared to control diet and this increase in dry matter remained concentration dependent. Maximum decrease in moisture content was observed in D\textsubscript{3} containing 500 FTU/kg phytase. Minimum decrease in moisture in the whole-body of the *Labeo rohita* was in the diet D\textsubscript{6} with 1250 FTU phytase. The results of our study are similar to those of Cheng and Hardy (2004) and Debnath et al. (2005) in which dry matter of fish increased significantly by increasing its digestibility co-efficient and meat quality of fish is also improved.

Table III shows that supplementation of different phytase levels increases the crude protein level in the whole body of the *Labeo rohita* (fingerlings). Greatest increase was observed in D\textsubscript{1} containing 500 FTU/kg phytase and the minimum increase was found in D\textsubscript{5} with 250 FTU phytase/Kg of the diet. In the same line of our study, the same observations were made in rainbow trout which is fed with the feed supplemented with 500 FTU microbial phytase/Kg of diet and Rohu fed with 1000 FTU phytase studied by Cheng and Hardy (2002), Rabia et al. (2017), and Debnath et al. (2005).

**Table III. Effect of different levels of phytase supplementation on moisture (%) in whole-body proximate of *Labeo rohita* (fingerlings).**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Phytase level (FTU/kg)</th>
<th>Moisture (%)</th>
<th>Crude protein (%)</th>
<th>Crude fat (%)</th>
<th>Crude ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0</td>
<td>69.91\textsuperscript{a}</td>
<td>19.4\textsuperscript{a}</td>
<td>5.7\textsuperscript{b}</td>
<td>2.63\textsuperscript{c}</td>
</tr>
<tr>
<td>D2</td>
<td>250</td>
<td>68.82\textsuperscript{b}</td>
<td>19.69\textsuperscript{b}</td>
<td>5.2\textsuperscript{a}</td>
<td>3.12\textsuperscript{b}</td>
</tr>
<tr>
<td>D3</td>
<td>500</td>
<td>68.38\textsuperscript{c}</td>
<td>20.45\textsuperscript{c}</td>
<td>5.19\textsuperscript{a}</td>
<td>2.99\textsuperscript{a}</td>
</tr>
<tr>
<td>D4</td>
<td>750</td>
<td>68.48\textsuperscript{d}</td>
<td>20.19\textsuperscript{d}</td>
<td>5.59\textsuperscript{a}</td>
<td>2.92\textsuperscript{a}</td>
</tr>
<tr>
<td>D5</td>
<td>1000</td>
<td>69.26\textsuperscript{e}</td>
<td>20.17\textsuperscript{e}</td>
<td>6.08\textsuperscript{a}</td>
<td>3.02\textsuperscript{a}</td>
</tr>
<tr>
<td>D6</td>
<td>1250</td>
<td>69.27\textsuperscript{f}</td>
<td>19.9\textsuperscript{f}</td>
<td>6.07\textsuperscript{a}</td>
<td>3.12\textsuperscript{a}</td>
</tr>
<tr>
<td>Pooles SE</td>
<td>0.03434</td>
<td>0.03379</td>
<td>0.02821</td>
<td>0.02769</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>P-value</td>
<td>P-value</td>
<td>P-value</td>
<td>P-value</td>
<td>P-value</td>
</tr>
<tr>
<td>Phytase</td>
<td>.0000***</td>
<td>.0000***</td>
<td>.0000***</td>
<td>.0001***</td>
<td></td>
</tr>
</tbody>
</table>

Values showing different superscripts in each row were statistically significant at \(p<0.05\).

Supplementation of different levels of phytase results in an increase in the crude fat and crude ash in proximate composition of *Labeo rohita* (fingerlings) (Table III). Highest increase in crude fat contents was found in D\textsubscript{3} containing 1000 FTU phytase per kg of diet. Minimum increase in crude fat was detected in diet D\textsubscript{6} with 500 FTU/kg phytase.

Highest increase of crude ash was in D\textsubscript{5} and D\textsubscript{6} containing 250 and 1250 FTU/kg phytase respectively. Minimum increase in crude ash was detected in diet D\textsubscript{4} with 750 FTU phytase/Kg of diet. Cheng and Hardy (2004) fed the rainbow trout with DDGS based phytase supplemented diet and showed an increased digestibility co-efficient value of ash content which is actually inorganic matter in fish represented as minerals that comes to be 7.3-99.7%.

**Conclusions**

Supplementation of phytase significantly (\(p<0.05\))
decreased the moisture contents (%) and increased the crude protein (%), crude fat (%) and crude ash (%) in the whole body of *L. rohita* fingerlings. Phytase supplementation improved the meat quality of *Labeo rohita* fingerlings.

**Statement of conflict of interest**

The authors have declared no conflict of interest.

**References**


