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EVALUATION OF NUTRIENT DIGESTIBILITY IN *LABEO ROHITA* JUVENILES FED LYSINE SUPPLEMENTED DIET

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ABSTRACT

A study was conducted in the laboratory conditions in the Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan during 2014 to investigate the effect of supplementation of lysine on nutrient digestibility in the juveniles of Labeo rohita. Seven experimental diets were formulated by supplementing 0, 0.5, 1, 1.5, 2, 2.5 and 3 lysine and designated as D1, D2, D3, D4, D5, D6 and D7, respectively. To test each diet, two replicates with 20 fish each (average weight 3.526±0.056g) were assigned. The experiment was performed within the time duration of 60 days. Water quality parameters like dissolved oxygen (5.8-7.3 mg/L), temperature (24.9-28.7°C) and pH (7.4-8.6) were maintained throughout this period. Results showed that lysine supplementation in fish diet increases the digestibility of crude protein and dry matter. Maximum digestibility of crude protein and dry matter was observed at lysine level of 1.5 and 2.5%, respectively. However, it was observed that digestibility of crude fat decreased in response to lysine supplementation. In conclusion, it was found that lysine supplementation improved the nutrient digestibility performance of juveniles of L. rohita

KEYWORDS: Labeo rohita; Rohu; fingerlings; Lysine; nutrient digestibility; Pakistan.

INTRODUCTION

The rate of utilization of fish meal in aqua feeds is increasing tremendously day by day due to increasing demand of aquaculture production (Forster and Ogata, 1998). Now a days, plant based meals are used to meet the protein and energy demands of aquatic animals because plant origin oils such as rice bran oil, peanut oil, soybean oil and linseed oil have good fatty acid profile which is necessary for fish (Masih *et al.,* 2014; Robinson *et al.,* 1980; Tahira and Butt, 2007). Sunflower seeds can provide Vitamin A and D, linoleic acid, oleic acid and unsaturated fatty acid (Gatlin *et al.,* 2007; Grewal and Williams, 2000). Plant based meal is the cheap source of proteins but has lower digestibility fish meal (Carter and Hauler, 2000), To overcome this deficiency, various dietary additives have been used to improve the nutritional value of plant meals-based diets including exogenous enzymes (Shah *et al.,* 2016, 2015a, 2015b), organic acids (Akram *et al.,* 2016; Shah *et al.,* 2016; 2015c), chelated mineral (Shah and Afzal, 2016) and vitamins (Fatima Shah *et al.,* 2016).

Plant based diet is also low in available protein and has poor essential amino acid profile (Tacon, 1995). Protein being the key nutrient, is required by organisms in huge amount in diet for proper functioning of their bodies (Lovell, 1989). It is the important structural component of bones. By weight, bone constitutes 22% protein as proteins play a key role in collagen formation. Fish require amino acid to strengthen its skeletal system (Hughes, 2003). Amino acid profile in the fish diet has an effect on dietary protein (Keembiyehetty and Gatlin, 1992). Lysine is one of the most important essential amino acid as many fish species had a considerable amount of lysine in their skeleton (Ahmed and Khan, 2004). Decreased growth and increased mortality are the major effects that can be seen in the fish fed with essential amino acid deficient diet (Ketola, 1983).

When the fish meal is replaced by the plant origin protein diet, major effect in almost all fin fish species is the deficiency of lysine in their bodies. Different fish species requires different amounts of lysine in their diet ranging from 3.2 to 6.2% (Fatima *et al.,* 2016; Harris, 1980; Ozorio *et al.,* 2003; Small and Soares, 2000; Wang *et al.,* 2005; Yang *et al.,* 2010).

Moreover, lysine plays an important role in controlling the biosynthesis of carnitine which takes place in liver and skeletal muscles. Carnitine assists in the transportation of long chain unsaturated fat molecules for β-oxidation in mitochondria which can serve as a fuel during times of fasting (Tacon, 1995). Lysine also helps in the maintenance of osmotic pressure and acid base balance. It is needed for the deposition of protein in the body (Chiu *et al.,* 1987, 1988). Previous studies have shown that lysine supplementation in diet also results in the improved weight gain of fish (Hu *et al.,* 2008; Robinson and Li, 1994; Robinson *et al.,* 1980; Viola

et al., 1992a; Walton *et al.,* 1984; Zarate and Lovell, 1997). The lysine requirement of fish is around 5.7% of its total dietary protein (Kim *et al.,* 1992; Anon. 1993; Singh, 1987; Wilson, 1985). Soybean meal-based diet may require supplementation of lysine due to high lysine requirement (57-70 g $Kg^{-1}$of protein) (Satheesha and Murthy, 1999; Ahmed and Khan, 2004).

Labeo rohita is widely cultured now a days due to its fast growth and disease resistance properties as it is considered as one of the tastiest fish among other fish species (Jhingran and Pullin, 1988). Accordingly, dietary lysine is essential in order to prepare nutritionally complete and cost-effective diet for intensive culture of fingerling *Labeo rohita*.

The purpose of present study was to determine the nutrient digestibility in *L. rohita* juveniles fed lysine supplemented diet.

MATERIALS AND METHODS

Labeo rohita juveniles (average weight 3.526±0.0056 g) were obtained from Government Fish Seed Hatchery, Faisalabad and transported to the Laboratory of Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan in the year 2014. These were kept in large tanks to acclimatize with the laboratory conditions. Then these were transferred to specially design V-shaped tanks (UA system) for the collection of fecal material from water media. Nine duplets were fed the respective test diets once in daily (Table 1). Water temperature (24.9-28.7°C), pH (7.4-8.6) and dissolved oxygen (5.8-7.3 mg/L) were monitored by the usage of thermometer, pH meter (Jenway, 3510), D.O. meter (Jenway, 970), respectively during the study period. Capillary system was used to provide aeration to all the tanks round-the-clock.

Table 1. Ingredients composition (%) of experimental diet

Ingredients	Composition (%)
Fish meal	17
Sunflower meal	25
Corn gluten meal	20
Soybean meal	15
Fish oil	7
Wheat flour	5.3
Mineral mixture*	3
Vitamin mixture**	3
$CaHPO_4$	2.5
Ascorbic acid	1
Chromic oxide	1
Choline Chloride	0.2
Total	100

Each Kg mineral granules contains
Ca (Calcium) 155gm, Mn (Manganese) 2000 mg, 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, **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

Experimental diets and feed ingredients

Ingredients were purchased from local poultry feed market. Prior to the formulation, chemical analysis of ingredients was carried out following AOAC (1995). Before adding in the experimental diets, the feed ingredients were ground and refined to required particle size (Table 2).

To prepare diets all dry ingredients were intermixed in electric mixer for 10-20 minutes; While mixing constantly, fish oil was gradually added during mixing. Inert marker (Chromic oxide 1%) was also incorporated during mixing. Lysine was added at the levels of 0, 0.5, 1, 1.5, 2, 2.5 and 3% to dry mixed ingredients to make seven test diets. Approximately 10-15% distilled water was added to the ingredient mixture to facilitate pelleting by lab extruder.

Table 2. Chemical composition (%) of experimental diets (Dry basis).

Diet	Lysine (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)
D1	0	91.905	30.849	8.7845
D2	0.5	91.565	30.9965	9.253
D3	1	92.29	31.264	9.1875
D4	1.5	92.71	30.701	9.112
D5	2	91.64	31.8945	9.7445
D6	2.5	92.225	31.194	8.9235
D7	3	91.89	31.694	8.6455

Sample collection and feeding protocol

Two replicates were assigned with stocking density of 20 fish for each test diet and diet was fed to the fish at satiation level, twice a day. Tanks were covered by net to prevent juveniles to jump out. After two hours of feeding session, the tanks were washed completely to remove particles of diet and refilled with fresh water. Fecal collection tube was used to collect faeces of each tank after two hours' gap. Fecal material of each replicated treatment dried in oven at 60°C, ground and stored for chemical analysis. The experiment lasted for 60 days.

Biochemical analysis

The samples of test diet and faeces were homogenized by pestle and mortar and analyzed by standard methods (AOAC, 1995). Oven was used to determine moisture contents by drying at 105°C for 12 H, micro Kjeldahl apparatus for crude protein after acid digestion, petroleum ether extraction method for crude fat through Soxhlet HT2 1045 system, crude ash, by burning at 65°C or 12 h in electric furnace (Eyela-TMF 3100) to constant weight.

Statistical analysis

The analyzed data of nutrient digestibility was subjected to statistical analysis using one way analysis of variance (ANOVA). The differences among means were tested by Newman Keuls Test and considered significant at $p<0.05$. Costate Computer Software, Version 6.303 was used for statistical analysis.

RESULTS AND DISCUSSION

The data (Table 3) illustrates that lysine supplementation significantly improved the dry matter digestibility upto 1.5% lysine level which was decreased with further supplementation. Similarly, crude protein digestibility was also increased by dietary supplementation upto lysine level of 2.5 %. However, dietary lysine supplementation showed no effect on crude fat digestibility.

Table 3. Effect of graded levels of lysine supplementation on apparent coefficient (ADC %) of nutrients in *Labeo rohita* juveniles.

Diet	Lysine level (%)	Dry matter	Crude protein	Crude fat
D1	0	72.62^{c}	89.67^{d}	85.07
D2	0.5	73.95^{a}	90.09^{cd}	86.32
D3	1	73.71^{ab}	90.39^{c}	86.36
D4	1.5	73.99^{a}	90.26^{c}	86.74
D5	2	73.26^{b}	91.19^{b}	87.98
D6	2.5	73.55^{ab}	91.71^{a}	87.42
D7	3	73.44^{ab}	90.73b^{c}	85.46
PSE		0.109	0.139	0.69
ANOVA				
***p*-value**		0.0006***	0.0002***	0.1628 ns

These observations also confirm the results of El-saidy and Gaber (2002) who observed that lysine supplementations in *L. rohita* significantly ($p<0.05$) improved the nutrient digestibility coefficients of crude protein, crude fat and gross energy with maximum increase at 0.5% L-lysine level. Similarly, Cheng *et al.* (2003) reported increased digestibility of crude protein in *Oncorhynchus mykiss* fed with 0.2% lysine supplemented diet. According to Williams *et al.* (2001), supplementation of amino acids in low protein diets improved the growth performance of barramundi *Lates calcarifer* juveniles and that only with low protein diet, amino acid were utilized efficiently by fish. It has been observed that supplementation of lysine in low protein diets increased the protein and lipid contents in the edible tissue (Zarate and Lovell, 1997). They also found that crude fat and dry matter digestibility were not affected by lysine supplementation. Some recent studies (Li *et al.,* 2014; Tang *et al.,* 2012) showed improved nutrient digestibility and growth performance in grass carp.

In contrast to our observations, Davies *et al.* (1997) observed no significant differences in the digestibility of crude protein, crude fat and dry matter in *Oncorhynchus mykiss* fed lysine supplemented diet. Similarly, Alam *et al.* (2005) also observed non-significant effect of lysine supplementation on the nutritional attributes in *Marsupenaeus japonica.* Moreover, Biswas *et al.* (2007), did not observe any significant effect of lysine supplementation on crude protein and crude ash digestibility in *Penaeus monodon*. Researches on the channel cat fish *Ictalurus punctatus* proved that lysine or methionine had no significant effect on growth performance of the fish (Webster *et al.,* 2000).

The differences in the lysine optimum levels for nutrient digestibility may be due to the differences in fish species, fish age and size, composition of basal diet, stocking density and culture conditions in the laboratory (Li *et al.,* 2014).

CONCLUSION

It is concluded that for *L. rohita* juveniles, the plant-based diet supplemented with lysine provided satisfactory nutrient digestibility performance when compared with the reference diet containing no supplementation. Hence, our results should encourage further studies on carp species for formulating low cost and more nutritive diet.

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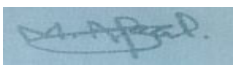
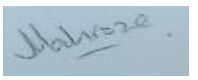
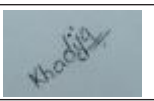
CONTRIBUTION OF AUTHORS

S. No.	Author name	Contribution	Signature
1.	Faryad Ali	Performed the experiment and collected the data	
2.	Muhammad Afzal	Supervised the experiment	
3.	Syed Zakir Hussain Shah	Helped in design of experiment and critically checked and improved the manuscript	
4.	Mahroze Fatima	Generated the idea, designed the study and wrote the manuscript	
5.	Khadija Akram	Helped in collection and analysis of data and writing of the manuscript	
6.	Rashid Hussain	Helped in improving the study and writing the manuscript