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# Amylase activity of fingerlings of freshwater fish *Labeo rohita* fed on formulated feed.

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**Abstract**
In the present study ninety days experiment was conducted in glass aquaria to study amylase activity in intestinal tissue of fingerlings of freshwater fish *Labeo rohita* fed on 100%, 75%, 50% and 25% non-conventional source of protein in formulation of feed i.e. Blood of bovine animals obtained from slaughter house and conventional feed i.e. groundnut oil cake. The fishes were fed at the rate of 2% of the body weight every day. After specific time intervals the fishes were weighed and sacrificed for intestinal tissue. The intestinal tissues were quickly excised and cleaned off extraneous material, weight and used for enzymatic estimation of amylase by Bern Field (1955) method. Results revealed that the amylase activity was highest at 75% followed by 50%, 25% and 100% formulated feeds as compared to conventional feed.

**Keywords:** *Labeo rohita,* Formulated Feed, Amylase, Nutritional analysis, Groundnut Oil Cake.

## 1. Introduction
Aquaculture feed industry is correlated to fish farming for development of aquaculture sector. Profitability of fish farming depends upon the sufficient consumption of feed. Fish meal is the conventional animal protein in fish diet and it contains balanced amino acids, vitamin content, palatability and growth factors [1, 2]. However the increasing cost of quality fish feed required for aquafeed, due to decline in stocks of fish form capture fishery and competition for feed in animal husbandry, there is need for search of alternative sources of animal protein for fish feed. Hence in the present study, non-conventional animal protein source as blood of bovine animal is used in feed formulation as primary ingredient along with other ingredient.
Development in aquaculture is strictly related to improvement in nutrition, and up gradation of fish husbandry practices, a challenge for future development in aquaculture. Mass scale fish production heavily relies on the amplification of proper feeding protocols to satisfy nutritional requirements of the cultured species. As the aquaculture industry grows, the need for specialized feeds designed for particular production system and species increases proportionately [3]. Thus, nutritionists and feed manufacturers have to concentrate their efforts on determining the feasibility and selection of wide variety of feedstuffs available to the feed industry for preparation of cost effective feeds. Presently appropriate quality fish feeds in desired quantity are not available. For this reason majority of people use their own farm made feeds in variety of shapes and forms [3]. Limited knowledge is available about nutrient requirements of different stages of fish and digestive processes in fish alimentary canal which is difficult to assess proper feed formulation for particular fish species. Taking into above considerations, selection of different ingredients from large expanse available in the market is really a challenging job which can ensure viable growth and guaranteed health, ultimately maintaining the economics of the fish business [3]. Feed taken by fish undergoes through several mechanical and chemical processes. The ability of fish to metabolize a diet depends on the availability of appropriate digestive enzymes, which mediate specific degradation pathways modulating both physical and chemical nature of foods [4].
Fish digestive enzymes emphasizing the mechanisms and best use of nutrients are of unquestionable importance as a background for the optimization of fish feeding procedures [5]. Moreover, the extension of such studies explore the role of many other factors in this

process; for example, age, type of feeding, diet composition in fish feed [6, 7, 8]. From the digestive enzyme profile point of view, it is possible to predict the ability of the species to use many different nutrients [9]. Amylase is a digestive enzyme that aids in the breakdown of carbohydrates by breaking the bonds between sugar molecules in polysaccharides through a hydrolysis reaction. It is important in the digestion of starch into sugars to make available energy sources for the body. Present study is aimed to determine effect of different combinations of formulated feed (100%, 75%, 50% and 25%) and conventional feed on amylase activity of freshwater fish *labeo rohita* which gives the understanding of utilization of nutrients in feed by fish.

## 2. Material and Method

### 2.1 Feed Formulation

In the present study, for formulation of fish feed, the non-conventional animal protein source such as blood of bovine animal (80 gm) was taken along with ingredients like milk powder (60 gm), corn flour (20 gm), and eggs (70 gm) were added and mixed well. Agar powder (4 gm) was added as binding agent and turmeric (0.5 gm) and garlic (1 gm) were added as antibiotics. Then the mixture was boiled, and cooled at room temperature. After cooling cod liver oil (3.5 ml) was added. The vitamin mixture of vitamin B complex (1 gm) and vitamin E (1 ml) were added in the mixture. This mixture was kept under refrigeration for 12 hrs. After 12 hrs it was squeezed over polythene sheet and then dried at room temperature for 24 hrs. The dried nodules were crushed into small pellets. The nodules were sun dried to avoid fungal infection. Finally it was weighted and stored in the bottle.

Following the above procedure all the feeds were formulated in the percentage composition of 25% ( blood 25% +groundnut oil cake 75%), 50% (blood 50%+groundnut oil cake50%), 75% (blood 75% +groundnut oil cake 25%), 100% formulated (100% blood) and 100% conventional (100% groundnut oil cake).

### 2.2 Proximate analysis of formulated feeds

The feed was analyzed for moisture [10] crude protein, crude lipid, crude fiber, and ash values. Crude protein was determined after acid digestion by Kjeldhal method [11] lipid after extraction with petroleum ether in a Soxhlet apparatus, dry matter after drying at 100-105 $^{o}$C for 24 hrs and ash after combustion at 550 $^{o}$C for 12 hrs. Gross energy values were calculated assuming the energy values of protein and lipid [12].

### 2.3 Experimental design

After acclimatization, 25 fingerlings (5-6 cm) of experimental fishes were randomly stocked in each aquarium for three months (90 days) in laboratory condition. This stocked experimental fishes were fed by conventional and non-conventional formulated feeds. The average initial weight (2-3 grams) of fingerlings was noted. During experiment, all physico-chemical parameters of experimental water (such as temperature, pH, alkalinity, hardness, dissolved oxygen, free carbon dioxide) were maintained within permissible limit. The fishes were fed at the rate of 2% of total body weight once a day. The body weights of fishes were recorded at selected time intervals (30, 45, 60, 75, and 90 days).

### 2.4 Enzymatic studies

After time intervals of 30, 45, 60, 75 and 90 days fingerlings of fishes, five from each group were taken, weighed and sacrificed for intestine. The intestinal tissue was quickly excised and cleaned off extraneous material, weighed and used for enzyme estimation. The tissue was homogenized and tissue concentration was made to 10 mg/ml with 0.09% chilled NaCl solution. The tissue homogenate was centrifuged at 3000 rpm for 10 minutes. Aliquot of supernatant was used as enzyme source. The homogenize was stored in freezer until used.

### 2.5 Amylase [13]

The assay mixture of amylase consists of 1 ml substrate, 1 ml of 0.1 M phosphate buffer of appropriate pH and 0.5 ml supernatant. The substrate used was 1% starch. The test tubes incubated at 40 $^{o}$C temperature for 10 minutes. The reaction was terminated by adding 2 ml DNSA followed by 2 ml distilled water. The test tubes were heated in boiling water bath exactly for 5 minutes. Then the test tubes were cooled. The colour was developed by direct reaction with maltose using DNSA reagent under similar assay conditions. The specific activity was expressed as mg maltose/100 mg wet tissue/hr.

### 2.6 Statistical analysis

Statistical analysis of data was performed by Analysis of Variance (ANOVA) using graph pad software. Value expressed is mean of n (n=5) ± SD, * P< 0.05, **P<0.01, ***P<0.001, NS = Non significant.

## 3. Results and Discussion

The development and secretion of digestive enzyme in fish is strongly dependent on nature and composition of feed which is administered to fish, as well as on metabolic capabilities of fish. Enzymes in fish are effective tool for identification of particular components of animal's diet [14].

Amylase activity has been reported in various herbivorous and omnivorous species due to its role in breakdown of polysaccharides into short-chain sugars [15]. Moderate amylase activities were reported in carnivorous species [16, 17]. In fish amylase is needed to digest glycogen, an energy source which is found in animal tissue.

From the present studies it is revealed that, conventional feed contains 33.94% of protein while the formulated feed had 43.27% of protein (Table 1). The total energy gained from formulated feed was 386.80 kcal/100 g while it was 372.82 Kcal/100 g for conventional feed.

The experimental fishes fed on formulated feed noted highest amylase activity at 75% formulated feed and it was 30.76±1.79 mg maltose/100 mg wet tissue/hr followed by 50% formulated feed, 30.68±2.25 mg maltose/100 mg wet tissue/hr, 25% formulated feed was 29.86±2.43 mg maltose/100 mg wet tissue/hr and 100% formulated feed was 29.66±1.4 mg maltose/100 mg wet tissue/hr respectively as compare to conventional feed 27.68±2.70 mg maltose/100 mg wet/hr (Table 2).

**Table 1:** Nutritional analysis of conventional and formulated feed.

| Sr. no | Parameter | Conventional feed | Formulated feed | | | |
|---|---|---|---|---|---|---|
| | | | 100% | 75% | 50% | 25% |
| 1 | Moisture (%) | 6.16 | 5.62 | 8.01 | 6.78 | 6.79 |
| 2 | Ash (%) | 4.73 | 6.12 | 3.64 | 3.69 | 4.39 |
| 3 | Protein (%) | 33.94 | 31.84 | 43.27 | 29.89 | 30.29 |
| 4 | Fat (%) | 7.72 | 4.01 | 7.46 | 6,52 | 5.4 |
| 5 | Fiber (%) | 2.28 | 6.05 | 8.8 | 6.2 | 2.4 |
| 6 | Carbohydrate | 44.64 | 52.34 | 50.23 | 52.18 | 51.47 |
| 7 | Energy kcal/100 g | 372.82 | 315.42 | 315.43 | 386.80 | 375.51 |

**Table 2:** Amylase activity from intestine of fingerlings of *Labeo rohita* fed on conventional and formulated feed

| Sr. No | Days | Conventional feed | Formulated feed | | | |
|---|---|---|---|---|---|---|
| | | | 100% | 75% | 50% | 25% |
| **1** | 30 days | 21.92±0.96 | 26.78±1.26* | 26.78±1.26* | 24.48±4.03$^{ns}$ | 22±1.20$^{ns}$ |
| **2** | 45 days | 30.12±4.19 | 33.14±2.44$^{ns}$ | 27.68±2.0$^{ns}$ | 30.68±2.66$^{ns}$ | 31.2±1.67$^{ns}$ |
| **3** | 60 days | 13.44± 2.68 | 21.68± 2.30 * | 23.2±1.23** | 23.68± 8.27** | 25.22± 1.58** |
| **4** | 75 days | 27.2± 0.76 | 28.8± 1.27$^{ns}$ | 27.16±1.00$^{ns}$ | 25.08±1.42* | 25.76±0.97$^{ns}$ |
| **5** | 90 days | 27.68±2.70 | 29.66±1.4$^{ns}$ | 30.76±1.79* | 30.68± 2.25$^{ns}$ | 29.68±2.43$^{ns}$ |

The highest amylase activity was found at 75% formulated feeds fed groups as compare to conventional feed. This might be due to formulated feeds contain the higher carbohydrate content than the conventional feed as well as good acceptability of feed by fish. Various workers have demonstrated that amylase activity is greater in omnivorous and herbivorous fish than in carnivorous fish [18, 19, 20, 21, 22]. It is noted that, the decrease of dietary protein and consequent reduction of some amino acids are responsible for the reduction in the amylase expression. In addition, increase of dietary lipids also impacts on the reduction of amylase activity of fish [23, 24].

## 4. Conclusion

Based on result and observation made throughout 90 days experiment, it is concluded that, fingerlings of freshwater fish *Labeo rohita* fed on different combination of formulated feed (100%, 75%, 50% and 25%) and conventional feed, amylase activity was maximum at formulated feed as compare to conventional feed i.e. the formulated feed is well utilized and digested by fish. Thus, formulated feed in the diet of fish will have to be intensified in order to formulate cost effective fish feed for better fish growth.

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