

## EFFECT OF DIFFERENT ARTIFICIAL FEEDS FORMULATED FROM LOCAL INGREDIENTS ON THE MEAT QUALITY OF INDIAN MAJOR CARPS

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### ABSTRACT

The effect of three isoproteineous (35 % crude protein) feeds, prepared from different ingredients, on the meat quality of major carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*) was investigated. Total 90 fingerlings of each fish species were randomly stocked with three replicate tanks of each treatment. Maximum quantitative protein concentration was recorded in *C. mrigala* (0.798 + 0.013 mg/ml) followed by *L. rohita* (0.666 + 0.001 mg/ml) fed with diet B, whereas diet C showed maximum protein concentration (0.725 + 0.015 mg/ml) in *C. catla*. Minimum protein concentration was recorded as (0.495 + 0.019, 0.567 + 0.003 and 0.564 + 0.036 mg/ml in *C. mrigala*, *L. rohita* and *C. catla* fed with diet A, respectively. The order of protein concentration in *C. mrigala* and *L. rohita* was diet A < C < B, whereas in *C. catla* it was diet A < B < C. The protein efficiency of diets A and B in *C. mrigala* and *L. rohita* were statistically different. In *C. catla* the protein efficiency of the diet A was significantly different (P<0.01) from diet B, and diet A exhibited non-significant (P>0.05) with diet C. The qualitative analysis depicted a maximum number of bands of isolated protein subunits in *C. mrigala* which were 16, 13 and 15, respectively, in diets A, B and C. In *L. rohita* 13, 11 and 12 bands of isolated proteins of different molecular weight was observed after the effect of diet A, B and C, respectively. The number of bands of isolated protein subunits in *L. rohita* were 13, 11 and 12 in case of diets A, B and C. In *C. catla* 12, 10 and 11 in bands of isolated proteins of different molecular weights were observed after feeding with A, B and C, respectively. The overall order of the number of bands of isolated protein subunits in all fish species was diet A > C > B.

**Key words:** fish; growth rate; feed efficiency; SDS-PAGE.

### INTRODUCTION

Fish is a vital protein source of human nutrition. Fisheries and fresh water are considered as one of the most promising source aquaculture for protein supply in the world. Fish flesh contains all the vital amino acids, minerals, and vitamins, especially A and D (Salim, 2006). It serves as an important source of protein to a vigorous diet due to its low carbohydrates and unsaturated fatty acid profile, particularly omega-3 contents (Razvi, 2006). Therefore, traditional diet with adequate addition of fish is considered best for the fish growth and quality of fish meat (Salim, 2006; Yildirim, 2008).

In Pakistan, successful composite fish culture system of major and exotic carps has been introduced (Mahboob and Sheri, 1998). A number of factors together with food, space, temperature, salinity, season and physical activity affect fish growth. Protein is the most significant nutrient for growth that constitutes the main component of the diet and usually the most expensive element in artificial feed. The quality and quantity of dietary protein strongly influence growth rate in fishes (Wilson and Halver, 1986). The role of supplementary feed in intensive fish farming cannot be overlooked as the complete nutritional requirements of fish depend upon the feed. Calcium, anti-hypertensive proteins,

antioxidants, selenium, chitin, -3 PUFAs, taurine and other bioactive components in fish and fish products provide them a prime importance (Gormley, 2006).

Fish proteins contain all the essential amino acids. Cereal grains are usually low in lysine and/or sulphur-containing amino acids (methionine and cysteine), whereas fish protein is an excellent source of these amino acids (Mahboob and Sheri, 1998). The amino acid composition of fish protein is such that it can provide the sole source of protein for humans, because of the presence and extent of the essential amino acids it contains. The actual amino acid pattern is comparable to that of other proteins of high Biological Value, such as beef, egg or milk protein. Fish collagen in general has, however, more amino acid contents than mammalian collagen (Grossman and Bergman, 1992). Fish meat is easily digestible and digestibility of fish meat is 85-95 % (Rudolph, 1971). The amount of connective tissue in fish and shellfish muscle is relatively low. It softens and dissolves more readily when heated compared to the connective tissue of land animals, and is readily hydrolyzed by digestive enzymes. It is, therefore, easy to chew and digest when cooked (Holland *et al.*, 1993). Fish myofibrillar protein has excellent functional properties such as emulsifying properties, gel-forming ability, and water-holding capacity (Hashimoto *et al.*, 1982). Fish has assumed great importance as a result of anti-cancerous

effects, minimized risk of heart ailments and consequently prolongs life expectancy. It is a significant source of vitamins A and D (Gerking, 1966).

Indian major carps consisted of three species having greatest economic importance. These species are catla (*Catla catla* Hamilton), rohu (*Labeo rohita* Hamilton), and mrigal (*Cirrhinus mrigala* Hamilton) (FAO, 1990; 2000). *Labeo rohita*, commonly called Rohu in Hindi, *ru* in Bengali and *rou* in Assamese is one of the most important among the three Indian major carp fish species for aquaculture in India (Debnath *et al.*, 2007). It is popular in Thailand, Pakistan, Bangladesh, Orissa, Uttar Pradesh, West Bengal, Assam, and the Konkan region of India. Rohu a non-oily/white fish is also very popular in the province of Punjab, Northern India (Froese and Pauly, 2005). Keeping in view the consumer preference and economic importance of the major carps in the country the present study was planned to assess the effect of different feeds on the quantitative and qualitative changes in the protein contents of meat of Indian major carps (*Cirrhinus mrigala*, *Catla catla* and *Labeo rohita*).

## MATERIALS AND METHODS

A three-month feeding trial was conducted in specially designed water circulating tanks to assess the effects of different fish feeds on meat quality of *Catla catla* (thaila), *Cirrhinus mrigala* (mori) and *Labeo rohita* (rohu). Fish were procured from Fish Hatchery, Faisalabad, Pakistan and transported live to the Fisheries Research Laboratory, Department of Zoology, GC University, Faisalabad, Pakistan.

**Preparation of Feed:** Three different diets i.e. animal origin diet, plant origin diet and mixed diet were formulated by using locally available ingredients (calf blood, intestine, liver and stomach for animal origin diet (Table 1) and corn flour, rice polish, soya bean meal and mustard oil cake, for plant origin diet (Table 1). Mixed diet was the mixture of both plant and animal ingredients (Table 1). The feed was prepared by following "Pearson Square method" to prepare three isoprotienous diets with a 35 % crude protein contents from the selected locally produced feed ingredients. Fish were fed once daily to satiation (3% body weight) at 9.00 a.m. to 10.00 a.m., daily feeding allowance was adjusted fortnightly based on the average weight of the fish

**Collection and stocking of fish:** Randomly collected 90 fingerlings of *C. catla*, (mean weight =  $5.86 \pm 0.20$ g), 90 fingerlings of *C. mrigala* (mean weight =  $5.40 \pm 0.11$ g) and 90 fingerlings of *L. rohita* (mean weight =  $5.91 \pm 0.15$ g) from the ponds of Fish Seed Hatchery, Faisalabad, were shifted to the circulating tanks of Fish Seed Hatchery, Faisalabad.

The fingerlings (10 each fish species) were randomly distributed among the three tanks with triplicates (already marked with animal, plant and mixed according to feed based) for polyculture at a stocking density of 30 fish/tank. The interspecies stocking ratios for *C. catla*, *L. rohita* and *C. mrigala* was 30:40:30. The feeding trial started on April 15, 2014 and terminated on August 14, 2014.

**Preparation of Fish Samples for protein quality of fish meat:** *Catla catla* (150-200g), *Labeo rohita* (150-200) and *Cirrhinus mirgala* (150-200) were procured for this study. The scales, fins and skin of each sample were removed after feeding for 12 weeks and samples were washed with distilled water. Each fish was dissected and cut into pieces. Their fillets were minced and used for protein extraction and isolation immediately or after storage at  $-80^{\circ}\text{C}$  in plastic wraps.

**Protein Extraction and Purification:** The muscle tissue pieces were rinsed with chilled Phosphate Buffer Solution (PBS, pH 7.3) and pat dry with a lint-free cloth. The tissue was cut into small pieces approximately 10-20 mg each, and placed in separate 1.5 ml labelled Eppendorf tube. Proportionate to the muscle tissue weight, quantity of the extraction buffer was added to the sample and thoroughly mixed with a small glass rod (0.5g tissue / 3.125 ml extraction buffer). The samples were placed in a boiling water bath for 10 minutes to accelerate both extraction and protein denaturation. Then the samples were placed on ice for 5 minutes, and centrifuged ( $14000 \times g$ ). The aliquots (30  $\mu\text{L}$ ) of the supernatant were prepared and were analyzed by SDS-PAGE.

**Quantitative & Qualitative Estimation of protein:** Proteins estimation was done with the fish meat samples by the Bradford method (1976). Qualitative analysis of proteins was performed by SDS-PAGE using the Discontinuous Buffer System of Laemmli (1970) for separation of proteins. The molecular weights of the dissociated polypeptide were determined by using molecular weight pre-stained protein standards "Fermentas PageRuler™, Sigma USA" containing 250, 130, 95, 72, 55, 36, 28, 17 and 11 kDa protein subunits. For the determination of molecular weights of the proteins gel doc apparatus and software of photocapt (Version 12.4) was used.

**Statistical analysis:** The data thus obtained was analyzed using ANOVA (2 factors CRD and 2 way) by using software Minitab 15.

## RESULTS AND DISCUSSION

**Qualitative Estimation of Proteins:** The proteins were extracted from *C. catla*, *L. rohita* and *C. mrigala* with

three different diets (A: animal based, B; plant based and C: mixed diets). The protein efficiency of diet A in *C. mrigala*, *L. rohita* and *C. catla* was recorded as  $0.495 \pm 0.01$ ,  $0.567 \pm 0.003$  and  $0.564 \pm 0.036$  mg/ml, respectively. Statically there was a non-significant ( $P > 0.05$ ) difference among three fish species. In case of diet B the protein extraction efficiency of protein in *C. mrigala*, *L. rohita* and *C. catla* was recorded as  $0.798 \pm 0.013$ ,  $0.666 \pm 0.001$  and  $0.622 \pm 0.015$  mg/mL, respectively. The efficiency of diet C in *C. mrigala*, *L. rohita* and *C. catla* was recorded as  $0.561 \pm 0.029$ ,  $0.576 \pm 0.008$  and  $0.725 \pm 0.015$  mg/mL, respectively. The efficiency of both the diets (B & C) showed the same pattern in three fish species such as, the diet C was significantly different ( $P < 0.01$ ) from Diet A & B whereas both the diets A & B showed non-significant difference ( $P > 0.05$ ). The two fish species, i.e. *C. mrigala*, *L. rohita* showed the same response for the protein efficiency of different feeds that the efficiency of diet A showed non-significant difference ( $P > 0.05$ ) from diet B and significant difference ( $P < 0.01$ ) from diet C. In case of *C. catla* the efficiency of the diet A was significantly different ( $P < 0.01$ ) from Diet B, whereas non-significantly different ( $P > 0.05$ ) from diet C (Table 2).

#### Qualitative Analysis of proteins by SDS-PAGE:

Several proteins were observed in muscle protein extracts of these fish species were run on SDS-PAGE against the markers (11-250 KDa). *C. mrigala* fed with diet A has more proteins (16) than diet B (13), whereas diet C showed 15 isolated proteins of different molecular weights. It was observed that *C. mrigala* fed with the diet of the isolated proteins have molecular weights viz., 11, 14, 16, 26, 32, 36, 45, 70, 74, 80, 84, 107, 139, 195, 244 and 260 KDa, respectively. The fish fed with diet B the isolated proteins have molecular weights (14, 17, 21, 23, 26, 35, 52, 65, 72, 85, 87, 102, 122, 180 and 249 KDa) whereas in the case of diet C the isolated proteins have molecular weights 13, 15, 18, 22, 29, 38, 68, 74, 87, 98, 115, 168 and 232 KDa (Table 3, Fig. 1).

In *L. rohita* fed with diet A, B and C, a variable number (13, 11 and 12) of protein bands were observed, respectively (Table 4). In the muscle of *L. rohita* fed with the diet A the isolated proteins have molecular weights 13, 15, 16, 17, 18, 21, 28, 62, 72, 75, 89, 128 and 202 KDa, respectively. In case of diet B the isolated proteins in muscle samples were observed as 15, 17, 18, 21, 27, 60, 72, 90, 110, 126, and 197 KDa. *L. rohita* fed with diet C the isolated the proteins of different molecular weights in the muscle were recorded as 13, 20, 24, 27, 45, 60, 71, 82, 93, 98, 122 and 202 KDa respectively (Table 4 and Fig. 2).

In the muscle samples of *Catla catla* fed with diets A, B and C 12, 10 and 11 protein bands were recorded, respectively (Table 5). In case of diet A the isolated proteins have molecular weights as 11, 14, 16,

23, 32, 36, 45, 84, 107, 139, 195 and 244 KDa, respectively. In case of diet B the isolated proteins have molecular weights as 11, 14, 19, 25, 42, 52, 85, 116, 185 and 246 KDa. In case of diet C the isolated proteins in the muscle samples have molecular weights ranging from 11-249 KDa, respectively (Table 3 and Fig. 3).

The three fish species fed with diet A shared three bands (21, 17, 12 KDa), only one sharing band (181KDa) in *L. rohita* and *C. catla*. Two bands were shared between *L. rohita* and *C. mrigala* (131, 26 KDa) and *C. catla* and *C. mrigala* (80, 63 KDa). In case of fish fed with diet B only one sharing band (72 KDa) between *L. rohita* and *C. catla* and three sharing bands (88, 30 and 18 KDa) between *C. catla* and *C. mrigala*. The fish species fed with diet C have four common bands (138, 30, 23, 16 KDa) between *L. rohita* and *C. mrigala*, three sharing bands (185, 82, 19 KDa) between *L. rohita* and *C. catla* and only one sharing band *C. catla* and *C. mrigala* (36 KDa) (Table 3).

In the present study, the impact of different feeds (based on local ingredients) on the qualitative and quantitative estimation of protein in the muscle of three fish species were in line with the findings of Ashraf *et al.* (2008) who reported that low cost based diet showed highest growth presentation and survival rate. The protein concentrations were more in these fish species fed with diet B comparison to diet A and C. Our results are in agreement with findings of Li *et al.* (2000). Islam *et al.* (2008) analyzed the endurance of carp fingerlings in terms of growth rate and harvested fish biomass by using an artificial diet comprising of rice bran, soya bean meal, fish meal, vegetable oil, vitamin and mineral mixture (40:20:10:3:2). The present findings were also inconsistent with Singh *et al.* (2005), who determined protein requirements for optimum growth with four diets having protein levels of 25-40% with slaughterhouse waste as the major protein source and hand fed to the fingerlings of *Labeo rohita* maintained under laboratory conditions.

The effect of diets on the concentration of proteins revealed that diets have a significant effect ( $P < 0.01$ ) on all three fish species and these findings are supported by the findings of Salim (2006), who reported higher growth of carps in natural ponds having the higher availability of natural food. Specific growth rate and body protein contents increased with higher dietary protein. Feed containing 33.32% dietary protein with a protein to energy ratio of 16.26 mg/kg appeared to be the optimal dietary protein for this species. In present study nebulin (107 KDa) is isolated and characterized by SDS-PAGE which is in close agreement with the study of Mitsuhashi *et al.*, (2002) and Fock and Hinssen (2002), who characterized nebulin as an integral part of skeletal muscle of fish. The findings of this study were in line with the findings of Okagaki *et al.*, (2005) and Montowska *et al.*, (2007) who reported that the molecular

weights of light chains of myosin of carp ranged between 16 and 26 KDa. The present study is also in line with findings of Mathew and Parkash (2006) who reported

myosin heavy and light chains having molecular weights of 205, 31, 23 and 22 KDa, respectively.

**Table 1. Percentage composition of feed ingredients of three different experimental feeds.**

| Feed I Animal Origin  |                  | Feed II<br>Plant origin |                  | Feed III<br>(Animal and Plant origin) |                  |
|-----------------------|------------------|-------------------------|------------------|---------------------------------------|------------------|
| Source of Ingredients | % of ingredients | Source of ingredients   | % of ingredients | Source of ingredients                 | % of ingredients |
| Calf blood            | 34.32            | Corn flour              | 33.63            | Calf blood                            | 17.16            |
| Intestine             | 30.19            | Rice polish             | 30.62            | Intestine                             | 15.16            |
| Liver                 | 19.36            | Soybean meal            | 19.82            | Liver                                 | 9.67             |
| Stomach               | 16.13            | Mustard cake            | 15.93            | Stomach                               | 8.07             |
|                       |                  |                         |                  | Corn flour                            | 16.81            |
|                       |                  |                         |                  | Rice polish                           | 15.31            |
|                       |                  |                         |                  | Soya bean meal                        | 9.91             |
|                       |                  |                         |                  | Mustard cake                          | 7.97             |
| Crude Protein         | 35%              |                         | 35%              |                                       | 35%              |

**Table 2. Protein conversion efficiency of three fish species fed with different experimental diets.**

| Specie            | Diet A                     | Diet B                     | Diet C                     |
|-------------------|----------------------------|----------------------------|----------------------------|
| <i>C. mrigala</i> | 0.495 ± 0.019 <sup>a</sup> | 0.798 ± 0.013 <sup>a</sup> | 0.561 ± 0.029 <sup>b</sup> |
| <i>L. rohita</i>  | 0.567 ± 0.003 <sup>a</sup> | 0.666 ± 0.001 <sup>a</sup> | 0.576 ± 0.008 <sup>b</sup> |
| <i>C. catla</i>   | 0.564 ± 0.036 <sup>a</sup> | 0.622 ± 0.015 <sup>b</sup> | 0.725 ± 0.015 <sup>a</sup> |

**Table 3. Isolated Protein bands in muscle samples of *Cirrhinus mrigala*, *Labeo rohita* and *Catla catla* fed three experimental diets.**

| <i>Cirrhinus mrigala</i> |        |        | <i>Labeo rohita</i> |        |        | <i>Catla catla</i> |        |        |
|--------------------------|--------|--------|---------------------|--------|--------|--------------------|--------|--------|
| Diet A                   | Diet B | Diet C | Diet A              | Diet B | Diet C | Diet A             | Diet B | Diet C |
| 16                       | 13     | 15     | 13                  | 11     | 12     | 12                 | 10     | 11     |

**Table 4. Characterization of Isolated Protein on the basis of their molecular weights in muscle of *Cirrhinus mrigala*, *Labeo rohita* and *Catla catla* fed with different diets.**

| <i>Cirrhinus mrigala</i> |        |        |        | <i>Labeo rohita</i> |        |        |        | <i>Catla catla</i> |        |        |        |
|--------------------------|--------|--------|--------|---------------------|--------|--------|--------|--------------------|--------|--------|--------|
| Mol. Wt.                 | Feed A | Feed B | Feed C | Mol. Wt.            | Feed A | Feed B | Feed C | Mol. Wt.           | Feed A | Feed B | Feed C |
| 250                      | 260    |        |        | 250                 |        |        |        | 250                |        |        |        |
|                          |        |        | 249    |                     | 202    |        | 202    |                    |        |        | 249    |
|                          | 244    |        |        | 130                 |        | 197    |        |                    | 244    |        | 246    |
|                          |        | 232    |        |                     | 128    |        |        |                    | 195    |        |        |
|                          | 195    |        | 180    |                     |        | 126    |        |                    |        | 185    |        |
|                          |        | 168    |        |                     |        | 110    |        |                    | 139    |        | 180    |
| 130                      | 139    |        |        | 95                  |        |        | 98     | 130                |        |        |        |
|                          |        |        | 122    |                     |        |        | 93     |                    |        | 116    | 122    |
|                          | 107    |        |        |                     |        | 90     |        |                    | 107    |        |        |
|                          |        |        | 102    |                     | 89     |        |        | 95                 |        |        | 102    |
|                          |        | 98     |        |                     | 75     |        |        |                    |        | 85     | 85     |
| 95                       |        |        |        | 72                  | 72     | 72     |        |                    | 84     |        |        |
|                          |        | 87     | 87     |                     |        |        | 71     | 72                 |        |        | 72     |
|                          |        |        | 85     |                     | 62     |        |        | 55                 |        |        |        |
|                          | 84     |        |        |                     |        | 60     | 60     |                    |        | 52     |        |

|       |    |    |    |       |    |    |    |       |    |    |    |    |
|-------|----|----|----|-------|----|----|----|-------|----|----|----|----|
|       | 80 |    |    | 55    |    |    |    |       | 45 |    |    |    |
|       | 74 | 74 |    |       |    |    |    | 45    |    |    | 42 |    |
| 72    |    |    | 72 | 36    |    |    |    |       | 36 | 36 |    |    |
|       | 70 |    |    | 28    | 28 |    |    |       |    |    |    | 35 |
|       |    | 68 |    |       |    | 27 | 27 |       |    | 32 |    |    |
| 55    |    |    | 65 |       |    | 21 | 21 | 24    | 28 |    |    | 26 |
|       |    |    | 52 |       |    |    |    | 20    |    |    | 25 |    |
|       | 45 |    |    |       | 18 | 18 |    |       |    | 23 |    |    |
| 36    | 36 | 38 |    | 17    | 17 | 17 |    |       |    |    |    | 21 |
|       |    |    | 35 |       | 16 |    |    |       |    |    | 19 |    |
|       | 32 |    |    |       | 15 | 15 |    |       | 17 |    |    | 17 |
|       |    | 29 |    | 11    | 13 |    |    | 13    |    | 16 |    |    |
| 28    |    |    |    |       |    |    |    |       | 11 | 14 | 14 |    |
|       | 26 |    | 26 |       |    |    |    |       |    | 11 | 11 | 11 |
|       |    |    | 23 |       |    |    |    |       |    |    |    |    |
|       |    | 22 |    |       |    |    |    |       |    |    |    |    |
|       |    |    | 21 |       |    |    |    |       |    |    |    |    |
| 17    |    | 18 |    |       |    |    |    |       |    |    |    |    |
|       | 16 |    | 17 |       |    |    |    |       |    |    |    |    |
|       |    | 15 |    |       |    |    |    |       |    |    |    |    |
|       | 14 |    | 14 |       |    |    |    |       |    |    |    |    |
|       |    | 13 |    |       |    |    |    |       |    |    |    |    |
| 11    | 11 |    |    |       |    |    |    |       |    |    |    |    |
| Total | 16 | 13 | 15 | Total | 13 | 11 | 12 | Total | 12 | 10 | 11 |    |

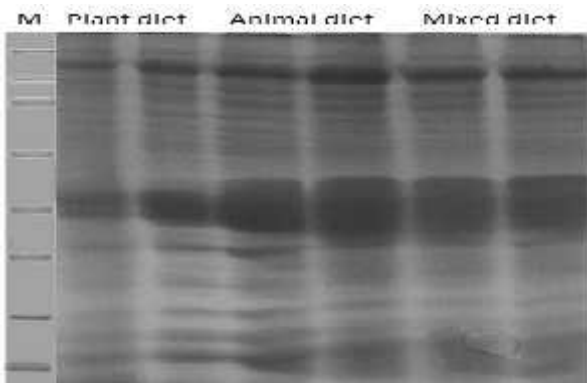


Figure 1. Gel photograph showing the isolated proteins in different diets in *Cirrihinus mrigala*

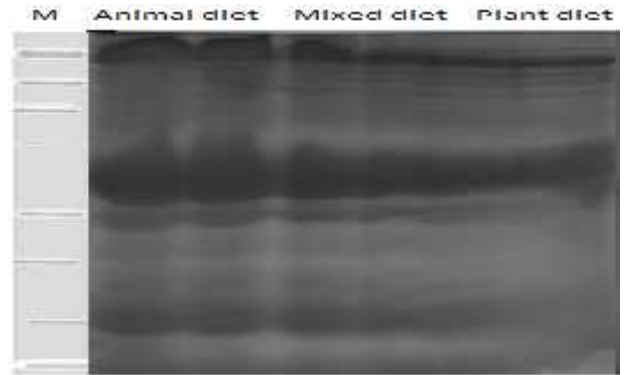


Figure 3. Gel photograph showing the isolated proteins in different diets in *Catla catla*

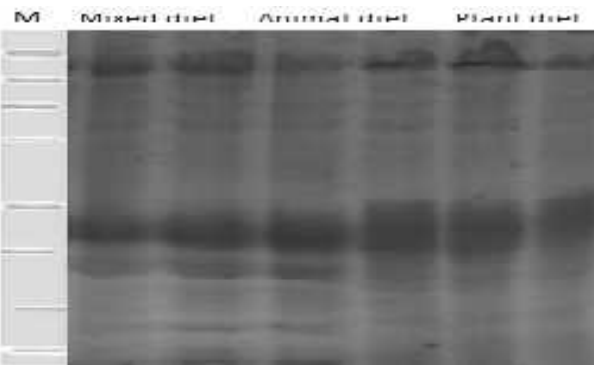


Figure 2. Gel photograph showing the isolated proteins in different diets in *Labeo rohita*

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