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CADMIUM TOXICITY STUDIES AND THE EFFECT OF SEVERAL BIO FEEDS IN *Labeo rohita* (HAMILTON 1822)

V. SIVABALAN [a*#] AND K. ARULDOSS [a#]

[a] Department of Zoology, Periyar Government Arts College, Cuddalore, Tamil Nadu-607001, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

The goal of this study was to compare and contrast the contents of proximate analysis, AST, ALT, LDH, and SDH in *Labeo rohita* gill, muscle, liver, and kidney. It is because of the high nutritional value of this fish as a source of protein in poor nations. The proximate study of *L. rohita* collected from various locations in Melarungunam, Cudalore district, Tamil Nadu found that hatchery *L. rohita* had the greatest protein (19.97%) and ash (1.76%) contents, whereas fat (0.84%), carbohydrate (5.39%), and dry matter (24.11%) contents. In the genus *Labio rohita*. The moisture content of the fish morphology was the greatest (81.42%). Maximum peroxidase and - amylase activity were measured during enzymatic analysis. The liver had the highest concentration of Cd metals, followed by kidney, muscle, and gills, in that order. When compared to other fish, rice bran and tapioca powder treated fish grew the fast.

Keywords: Aquaculture; enzymes; proximate analysis; antioxidant enzymes; cadmium; *Labeo rohita*. L.

1. INTRODUCTION

Modernization, industrialisation, and fertilisation are now the leading causes of ecosystem pollution, which is a severe problem all over the world. Toxic heavy metal contamination of water and soils is a severe environmental issue for which most traditional treatments do not give satisfactory remedies; in any event, most solutions are invasive and costly [1]. Cadmium (Cd) is a very poisonous nonessential transition metal that may harm people and animals. It is a contaminant that occurs naturally in the environment and is derived from agricultural and industrial sources [2]. Cadmium is largely absorbed by the consumption of contaminated food and drink, as well as, to a lesser extent, inhalation and cigarette smoking [3]. Cadmium is a heavy metal that accumulates in plants and animals and has a half-life of around 50 years. Beginning in 1912, cadmium poisoning was a severe problem in Toyama Prefecture (Japan), where many people ate rice cultivated in Cadmium contaminated irrigation water.

[#]*PG and Research*
**Corresponding author: Email: Sivabalan2593@gmail.com;*

Cadmium poisoning in fish causes tissue damage and malfunction (especially in the kidney and spleen), as well as haematological problems. Haematological alterations are widely employed as a measure of a fish's physiological and health state [4]. Trace element concentrations are hazardous not only to fish development and reproduction, but also to humans. Minerals have an important part in osmoregulation, intermediate metabolism, and the creation of the skeleton, healthy scales, teeth, and bones [5].

Rohu is a carp species that lives in freshwater and may be found in rivers across Asia (India, Bangladesh, Burma Pakistan, and Nepal). India. The *Labeo rohita* fish species is predominantly found in the provinces of Tamil Nadu and Punjab, and it is commonly used as food due to its nonoil character. Fish is an excellent source of a well-balanced diet. Fish proteins have a high biological value and include all of the required amino acids, as well as lysine, which is scarce in vegetarian meals [6]. Fish tissue, notably the gill, muscle, kidney, and liver, possess antioxidant defence mechanisms such as catalase, superoxide dismutase, and peroxidase, among others, to protect themselves from the oxidative effects of heavy metals [7].These enzymes act as a first line of defence against oxygen, derived free radicals, preventing oxidative stress in fish tissues [8]. Antioxidant enzymes, on the other hand, have varying activity in saltwater and freshwater fish cells, tissues, and organs, depending on the feeding habitat, ambient circumstances, and other ecological factors.

Labeo rohita is a promising aquaculture species in India and other Asian nations since it is an emerging fisheries resource with a high viability. There is a scarcity of information on antioxidant enzymes and nutritional profile in relation to heavy metal stress in this species. As a result, this study was designed to analyse several biochemical parameters in *L. rohita*, including as proteins, lipids, carbohydrates, and chromium of various enzymes.

2. MATERIALS AND METHODS

Freshwater fish *Labeo rohita* were gathered from commercial fish farms in and around Cudalore, Tamil Nadu, India, between June and October 2019. The fish were brought to the lab alive, acclimated, and raised for 7 days in a glass tank (75 24 40 cm). The fish were divided into two groups: control and experimental, each with 20 fish, one fed a formulated diet and the other fed a live feed organism mixed diet. An aerator was used to provide continuous aeration. Every other day, the water in both aquaria was changed. The studies were repeated every 15 days for a total of 55 days.

2.1 Moisture, Crude Protein, Total Fat, and Total Ash were measured using the Methods provided by the Association of Official Analytical Chemists [9]

The reduction in weight of fish meat samples was used to compute the moisture percentage. Meat samples were used to quantify total fat and ash.

2.2 Determine Total Fat [9]

Percentage of Total fat = (Weight of fat × 100 Weight of sample / Wt., of Sample)

2.3 Heavy Metal Classification

The sample and 10ml concentrated HNO3 were added to a 100 ml tube and cooked on a hot plate at 100, 150, 200, and 250 degrees Celsius for 0.5, 0.5, and 1.5 hours, respectively. After that, HClO4 was added. After that, we added 2 mL of 1N nitric acid and cooked the sample on a hot plate until it was entirely digested and translucent. The digested samples were then transferred to volumetric flasks (50 mL) with deionized water to make up the volume. The samples were filtered using a 0.45 m filter. The samples were filtered via a Millipore membrane filter with a 0.45 m pore size (Type HV). The metal Cadmium content of the filtrate was determined using an Atomic Absorption Spectrophotometer Analyst 400-Perkin Elmer, as described by Andale *et al.* [10].

2.4 Proximate Analysis

2.4.1 Moisture content estimation [9]

The weight loss that happens when a sample is dried to a constant weight in an oven is used to determine moisture. A feed sample of around 2g was weighed into a silica dish that had previously been dried and weighed. The sample was dried for 36 hours at 650°C in an oven, then cooled in a desiccator before being weighed. The drying and weighing process is repeated until the weight is consistent.

% Moisture = (wt of sample + dish before drying-wt of sample+ dish after drying / Wt. of sample taken
) x100

Ingredients and feed are generally compared for their nutritional content on a moisture-free or dry matter (DM) basis since the water content of feed varies so much.

% DM = 100 - % Moisture.

2.4.2 Protein estimation [11]

In the digestion flask, one gram of material was collected. 10 gm potassium sulphate, 1gm anhydrous sodium sulphate, 1gm cupric sulphate, 1gm mercuric oxide, a grain of selenium dioxide, and 20 ml concentrated sulphuric acid were added to this. After that, the digestion tubes were placed in the digestion chamber, and the sample was digested for about three hours, until the solution became clear. This digested sample was distilled with 40 percent sodium hydroxide solution containing 40 ml of 4 percent boric acid and two drops of mixed indicator after being given 20 ml of water. The steam from the distillate sample was collected in a flask and titrated against a solution of 0.1N HCl.

Nitrogen (%) = A ´ 1.4007 ´ Strength of the titrant / S

Where,

A = Sample titrate reading.
S = Sample weight.
Protein content (%) = N × C

Where,

C is the conversation factor and
C = 6.25

2.4.3 Total ash estimation [11]

Four gram of the material were weighed into a shallow, reasonably broad washing dish (silica crucible), which was then fired in a furnace at 800°C for five hours (dull red) until light grey ash resulted or the weight was constant. Then it was allowed to cool before being weighed at room temperature.

Ash content (%) = B – A / S × 100

Where,

A = Weight of empty crucible silica
B = Weight of silica crucible with ash
S = Weight of sample

2.4.4 Estimation of crude fibre

In a 500 ml beaker, four gram of defatted material were weighed and added to 200 ml of 1.25 percent Sulphuric acid. On a hot plate, the beaker was boiled for 30 minutes. Any volume lost during the boiling process was made up with distilled water. The residues were rinsed with distilled water after the heated solution was filtered through a cotton towel. 200 mL of 1.25 percent sodium hydroxide solution was added to the residual and heated for 30 minutes. The residue was filtered through a cotton towel and rinsed with distilled water until it was no longer alkaline. The residue was dried for three hours at 105°C before being weighed again.

Crude fibre (%) = B – A / S × 100

Where,

A = Weight of empty dish
B = Weight of fibre sample with dish
S = Weight of defatted sample

2.4.5 Carbohydrates estimation [12]

100 mg of powdered materials were placed in a test tube and hydrolysed for 30 minutes at 1000C with 2 ml of concentrated H2SO4. 1 ml of 5 percent phenol and 5 ml of Conc. H2SO4 were added to 0.5 ml of hydrolysed and carefully mixed. A spectrophotometer was used to measure the colour development at 490nm (U2001Hitachi). The carbohydrate content of the samples was estimated by comparing the absorbance value to a glucose reference.

2.4.6 Fat estimation [9]

In a paper thimble, one gram of crushed and dried material was placed in a fat extractor flask that had been reweighed. The flask was filled with 80 mL of petroleum ether, which was then refluxed for 8 hours. In a desiccator, the flask was chilled, and the weight of crude fat removed was measured. Using a formula, the % crude fat was calculated.

Calculation:

Weight of flask with fat - weight of empty flask Crude fat (%) = ×100 / Weight of original sample

2.5 Enzymes

Like AST, ALT, LDH, and SDH were tested using conventional enzymatic procedures.

2.6 Analytical Statistics

The analysis of variance was used to determine statistical differences between variables. The data were presented as mean standard deviation, with p values of 0.05 considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Proximate Examination of Samples of Fish Flesh

The table below shows the results of the proximate analysis of fish flesh samples. 1. Hatchery *L. rohita* had the highest protein content (19.97 percent), but farmed *L. rohita* had lower protein content (13.76 percent). The overall fat content of river fish was highest (0.84 percent) and lowest in farmed fish (0.64 percent). Fish lipids are the most significant elements that define the quality of the fish flesh. Fish fat content varies by species and is affected by seasonal fluctuations, physiology, eating, habitats, and other factors. Carbohydrate content was measured in hatchery fish (1.97 percent) and river fish (5.39 percent), respectively. The greatest levels of ash and moisture were reported in hatchery and farmed fish, respectively. Farmed fish had the highest dry matter value (24.11 percent) and the lowest (18.57 percent). A variety of parameters, including size, weight, and feed consumption, determine the body composition of *L. rohita* (Table 2). The literature describes the standard values and maximum ranges of heavy metal concentrations (g/g wet weight) in fish (Table 1).

3.2 Analyzing Fish Flesh Samples in a Proximate Manner

As previously stated, the highest protein content of fish was attributable to good dietary ingestion and conversion into protein in the fish meat (Mahboob *et al.,* 2004). It was discovered that when fish body weight increased, the protein content increased significantly. Carbohydrates are vital as well, however they are present in fish in very little levels. When looking for lipids and protein content in fish, the percentage of moisture is a reliable indication. The higher the fats and protein concentration, the lower the moisture content (Dempson *et al.,* 2004). According to Vladau *et al*. [6], fish contains between 13 and 25 percent protein, accounting for 80 to 90 percent of the total protein content.

In different meals on rohu fish, the levels of AST content in the tissues of *Labeo rohita* combined with Cadmium over 30 days for gill muscle, kidney, and liver were examined. The highest levels of AST were seen in rice brawn and tapioca mixed organic feed fed fish, respectively. When comparing Cadmium treated and control fish, the lowest AST levels were found in Cadmium treated fish. Abdaleep *et al* (2008) and Abdaleep *et al* (2008) both reported and documented similar findings. The kidney and liver of fish contain antioxidant defence mechanisms to protect them from heavy metal oxidative stress [7]. Depending on ecological circumstances, feeding habitat, and other environmental variables, peroxidase activity varied among cells, tissues, and organs [13].

The liver is a key tissue in the investigation of the role of peroxidase in lipid peroxidation prevention because it is a significant site of detoxification and the primary target of ingested oxidants. Velma and Tchnounwou (2010) attempted to assess the organ specific harmful effects of hexavalent chromium in freshwater goldfish kidney and liver after acute exposure. In comparison to the control, their findings showed that peroxidase activity in both the kidney and the liver increased and changed significantly. The activity of peroxidase in the liver of G. brasiliensis was studied by Bray and Bettger [14], who discovered that the presence of zinc and copper powerful activators affects enzyme activity.

In different meals on rohu fish, the levels of AST content in the tissues of Labeo rohita combined with Cadmium for 30 days for gill muscle, kidneu, and liver were examined. The highest levels of AST were seen in rice brawn and tapioca mixed organic feedfed fish, respectively. When comparing Cadmium treated and control fish, the lowest ALT levels were found in Cadmium,treated fish. In Vladhu et al (2008) and Ahmed et al (2010), similar findings were observed and documented. Several environmental parameters, including as temperature.

Salinity, season and feeding areas, age, and sex, can have a significant impact on enzyme activity. Shen *et al.* [15] measured catalase activity in carp liver during pollution exposure, and the results revealed that there is an increase in catalase activity.

Table 1. Shows the standard values and maximum ranges of heavy metal concentrations (g/g wet weight) in fish documented in the literature

Organization/Country	Heavy Metal
	Chromium
FAO [16,17]	30
FAO/WHO limits (1989)	30
Turkish guidelines (Dural *et al.*, 2007)	20
Range of metal in present study	0.313-13.17

All tissue concentrations are in μg/g wet weight

Table.2 Proximate analysis rohu (*Labeo rohita*) for 30 days

Treatments	Parameter in (%)						
	Moisture	**Ash**	**Protein**	**Fat**	**Carbohydrate**	**Fibre**	**Energy**
T_1	83.56±2.56	16.32±0.48	19.97±0.59	0.84±0.02	1.97±0.59	18.57±0.04	25.78±0.77
T_2	65.58±1.96	12.56±0.37	13.76±0.41	0.59±0.01	1.32±0.09	19.43±0.582	18.21±0.54
T_3	85.01±2.55	18.76±0.56	21.87±0.65	0.91±0.02	1.98±0.05	19.55±0.586	26.26±0.78
T_4	86.32±2.58	19.45±0.58	22.15±0.66	0.93±0.02	2.00±0.06	20.11±0.60	27.02±0.81
T_5	87.09±2.61	21.98±0.65	25.78±0.77	1.22±0.03	2.34±0.69	22.34±0.67	29.77±0.89

± Standard Deviation; T_1-Control,T_2-Cadmium@100%,T_3-Rice brawn, andT_4- Tapioca powder, andT5-Rice brawn+ Tapioca powder,

Table 3. The levels of AST content in the tissues of *Labeo rohita* combination with Cadmium for 30 days

S. No	Tissues	Enzymes	Control	Cadmium	Rice brawn	Topioca	Rb+T
1.	Gill	AST	0.19±0.005	0.53±	1.53±0.015	1.79±0.053	2.34±0.07
2.	Muscle	AST	0.07±0.002	0.41±	0.93±0.27	1.34±0.040	1.54±0.04
3.	Kidney	AST	0.06±0.001	0.71±	1.23±0.03	1.54±0.045	2.91±0.087
4.	Liver	AST	0.05±0.000	076±	1.61±0.048	1.60±0.018	3.17±0.095

± Standard Deviation; Values are expressed as µ mole ALT/g wet wt. of the tissue.

Table 4. Levels of ALT content in the tissues of *Labeo rohita* treated with Cadmium and fish feeds in combination for 30 days

S. No	Tissues	Enzymes	Control	Cadmium	Rice brawn	Topioca	Rb+T
1.	Gill	ALT	0.09±0.002	0.48±0.01	1.23±0.036	1.59±0.04	1.78±0.05
2.	Muscle	ALT	0.06±0.001	0.40±0.01	0.73±0.021	1.34±0.39	1.32±0.03
3.	Kidney	ALT	0.05±0.001	0.65±0.01	1.03±0.001	1.32±0.03	1.56±0.04
4.	Liver	ALT	0.04±0.001	0.56±0.01	1.31±0.03	1.50±0.45	1.99±0.59

± Standard Deviation; Values were expressed as µ mole ALT/g wet wt. of the tissue.

Fig. 1. Treated fish of *Labeo rohita* L.

Fig. 2. Analytical activities of *Labeo rohita*

In varied meals on rohu fish, the levels of LDH and SDH content in the tissues of *Labeo rohita* combined with Cadmium over 30 days for gill muscle, kidney, and liver were analysed. Rice brawn and tapioca mixed organic feed ingested fish had the highest LDH SDH levels, respectively. When comparing Cadmium treated fish to control fish, the lowest levels of LDH and SDH were found. Basha and Rani [7] and Shen *et al.* [15] both reported and documented similar findings. The kidney is a vital organ in the body, and in addition to excretory tasks, it is responsible for maintaining homeostasis and erythropoiesis [18,19,20].Cadmium causes pathological alterations in the kidney, liver, muscle, and gills, including tubule damage and disorganisation, as well as glomerular oedema and necrosis [18,16 ,17].

The spleen is a haematological organ that serves various tasks. It's also the starting point for the removal of aged blood cells [21]. The digestive system modifications of several fish species show a relationship with their nutrition. This might be because their dietary components change in both habitats [22,23].

Cadmium is absorbed mostly through the respiratory system and to a lesser amount through the gastrointestinal tract, with skin absorption being uncommon. Cadmium enters the body through erythrocytes and is carried into the circulation .Cadmium is slowly excreted from the body through the kidneys, urine, saliva, and milk during breastfeeding. Cd exposure in humans can cause renal and hepatic dysfunction, pulmonary oedema, testicular injury, osteomalacia, and damage to the adrenals and haemopoietin system, among other things [24,25]. Similar findings were observed and documented [3,26,27]. There was also a link discovered between Cd exposure indicators (blood and urine) and coronary heart disease, stroke, peripheral artery disease, and thermogenic lipid profile abnormalities [28,29,30]. Cadmium is a confirmed human carcinogen (group I of the International Agency for Research on Cancer classification) in addition to its cytotoxic effects that

might cause apoptotic or necrotic processes [31,16,17]. Cadmium induces necrosis of pancreatic cells and fatty deposition in the spleen.

4. CONCLUSION

Heavy metal concentrations were found to be within the usual range for Cd, according to the research. The dose of a harmful metal obtained from fish, on the other hand, is determined not only by the concentration of certain metals in fish, but also by the amount of fish ingested. Although heavy metal levels are low, caution should be exercised since some people consume considerable amounts of fish on a daily basis. In general, the build-up of heavy metals in farmed fish was not as significant as it was in wild and hatchery fish, and it was judged appropriate for consumers in the current study. The concentration of heavy metals in the kidney was significantly higher than in other tissues. The amount of metal accumulated was different.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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