PROXIMATE ANALYSIS OF FISH *(Cirrihinus mrigala)* FED WITH DIFFERENT PROTEIN SOURCE DIETS

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A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2022

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

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This is to recommend that the thesis entitled, **"Proximate analysis of fish (*Cirrihinus mrigala*) fed with different protein source diets"** has been carried out by Ambika Paudel for the partial fulfillment of Master's Degree of Science in Zoology with a special paper Fish Biology and Aquaculture. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institution.

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LETTER OF APPROVAL

On the recommendation of the supervisor "Reader Dr. Archana Prasad" this thesis submitted by Ambika Paudel entitled **"Proximate analysis of fish (Cirrihinus mrigala) fed with different protein source diets"** is approved for the examination in partial fulfilment of requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ambika Paudel entitled **"Proximate analysis of fish (*Cirrihinus mrigala*) fed with different protein source diets"** has been accepted as partial fulfilment for the requirements of Master's Degree of Science in Zoology with a special paper Fish Biology and Aquaculture.

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Ambika Paudel

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LIST OF ABBREVIATIONS

Abbreviated forms	Details of abbreviations
AOAC	Association of Official Analytical Chemists
CL	Crude Lipid
CP	Crude protein
CGM	Corn gluten meal
CFPCC	Central Fisheries Promotion and Conservation Centre
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FM	Fish meal
MBM	Meat and bone meal
MOC	Mustard oil cake
NARC	National Animal Nutrition Research Centre
PBM	Poultry byproduct meal
PUFA	Polyunsaturated fatty acid
SBM	Soyabean meal

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ABSTRACT

An experiment was carried out to evaluate the proximate analysis of *Cirrihinus mrigala* by feeding different protein source diets. An experiment was conducted at Central Fisheries Promotion and Conservation Centre (CFPCC) from October 2021 to January 2022, Balaju. Fry of *Cirrihinus mrigala* of different length and weight 5.06 ± 0.5 cm and 1.5 ± 0.25 gm was kept in 12 hapas, 50 fry in each hapas. Three different diets with soyabean meal, mustard meal and fish meal were formulated and commercial meal was used as a control diet for the fry. For proximate analysis, fish of different length and weight 7.28 ± 0.5 cm and 3.20 ± 0.89 gm was collected for analysis from the treatments. Proximate analysis was performed in the laboratory of National Animal Nutrition Research Centre (NARC). For proximate analysis Association of Official Analytical Chemists (AOAC) method was used. At the end, carcass body composition of fish high moisture(%) 98.56 ± 0.59 was in mustard fed meal and low in commercial fed meal 96.97 ± 1.50, Ash (%) was high 18.42 ± 1.28 in soyabean fed meal and low 17.48 ± 0.89 in fish meal fed fish, protein (%) 68.45 ± 3.43 was high in commercial fed fish and low 57.07 ± 2.01 in soyabean fed fish and high lipid (%) 17.88 ± 2.46 was observed in soyabean fed fish and low 9.57 ± 1.62 in fish meal fed fish. Analysis was carried out in triplicate. At the end of the study, no significant difference was observed in moisture and ash but protein and lipid content shows significant differences. So, further studies with various alternative protein source are needed for conclusive information and knowledge for better proximate analysis of fish.

1. INTRODUCTION

1.1 Background

Approximately 50% of the fish production is globally for human consumption, making aquaculture one of the fastest expanding fields of the food industry (FAO 2012). Fish is regarded as a valuable and abundant source of protein that is both inexpensive and nutritious. In several Asian countries, fish cover almost 50 % of all animal protein consumption. High nutritional content fish tissue is advised as part of a healthy diet. Due to the rising demand for fish resources brought on by the rapid growth of the human population, fish species that could produce more biomass faster than native species were introduced (Begum et al. 2012). It is regarded as a wholesome source of both animal protein and vitamins. It also provides polyunsaturated fatty acids (PUFAs) and minerals required by the body for optimal health (Jim et al. 2017). The principal proteins found in muscles are myofibrillar proteins, sarcoplasmic proteins, connective tissue, stroma proteins, polypeptides, nucleotides, and nitrogen compounds that are not proteins. Along with vitamins A and D, fish is a good source of group B vitamins (Żmijewski et al. 2006). As lean fish is considered the best source of iodine, fatty fish are generally considered to be a good source of fatty acids (particularly EPA and DHA) and fat soluble vitamins (Efsa 2014, Skåre et al. 2014). Additionally, it is crucial for preventing cardiovascular diseases and lowers blood fats, notably triacylglycerides (Ahmed et al. 2015). Including fish in your diet can help with food security, cardiovascular health, and other health-related issues (Bezbaruah & Deka 2021). However, because of its short lifespan and rapid perishability, fish's nutritional benefits are reduced.

Fish meat is a good source of both macronutrients and micronutrients. Macronutrient constituents are protein, fat, ash, and carbohydrate whereas micronutrient constituents are fat-soluble vitamins (A, D, E, and K) and water-soluble vitamins (B complex and vitamin C) and minerals (calcium, sodium, potassium, magnesium, iron, copper, and selenium). Micronutrients are dietary components which are required in very small amounts and must be supplied from external sources (Lilly et al. 2017). Long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA) are present in fish lipids, and eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are

particularly important for human nutrition and health (Ahmed et al. 2022). Compared to other sources of animal protein, the flesh of fish contains a high quality of protein that is easy to digest (Louka et al. 2004).

The term “proximate composition” is the measure of the body weight percentage of each major biochemical component that makes up body mass, namely moisture, protein, lipid, carbohydrates and ash (Shearer 1994). A fish's body composition is a reliable predictor of its physiological state and water content in the body provides information on its relative levels of protein, fat, and energy. Because of size, sex, the feeding season, and the physical state of the fish being raised, there may be variations in the value of proximate composition both within and between species (Aberoumad & Pourshafi 2010). However, information about the chemical composition of freshwater fish is useful to nutritionists concerned about low-fat and high-protein diets like freshwater fish and to food scientists working to develop high-protein foods, assuring the best flavour, odour, texture, and nutritional value for consumers (Naeem et al. 2016). Knowledge of the fish's proximate composition is crucial for improved processing and preservation as well as knowing its nutritional worth (Begum et al. 2012). Fish nutritional value is influenced by the quality of the protein sources utilized in the feed composition (Luthada-Raswiswi et al. 2021). For the creation of enzymes, antibodies, and certain hormones, protein is a crucial food class. In addition to providing fatty acids, vitamins, and a portion of the energy needed to carry out everyday tasks, fat also serves as a source of energy (Herawati et al. 2017).

1.2 *Cirrihinus mrigala* (Hamilton, 1822)

Indian major carp include *Cirrihinus mrigala*. The ray-finned fish species known as "mrigal" belongs to the carp family. The body is smooth and bilaterally symmetrical, with cycloid scales covering it. South Asian rivers and streams are native habitats for this fish species. Although they are freshwater species, they can endure high salinity levels. The back of the body often has a dark grey shed, while the sides and belly are silvery. They are bottom feeders that replace dying plants and are benthopelagic and potamodromous plankton feeders. They primarily eat debris and have a limited range of fruit types. Bottom-feeding mrigal fish reach maturity at 1-2 years of age. It is frequently raised in polyculture alongside other carp. Fish called mrigal grow quickly (Iqbal et al. 2014). *Cirrihinus mrigala* is frequently consumed and has attracted a lot of

interest from aquaculturists worldwide due to its high production and delicious meat quality (Garibaldi 2012).

1.3 Protein Source

Protein is vital in fish diets because it provides both required and optional amino acids, which are important for enzymatic activity, the development of muscles, and the provision of energy for maintenance (Yang et al. 2002). Protein has a significant role in fish diets because of its effects in the growth and price of the diet (Jahan et al. 2013). In fish feed production, protein is the most expensive nutrient which contributes a major part of the total feed cost (Rahman et al. 2017). The optimization of fish production is important for the study of feeding methods because supplemental feeding is a key strategy for increasing fish productivity. Fish feed formulae are directly impacted by the growing demand for fish for human consumption because aquaculture needs to produce a lot of fish to meet demand. About half of a fish farm's operating expenses are covered by fish feed (Montoya-Camacho et al. 2019). Even if intensive fish culture produces quality fish feed made from locally available, less expensive plant and animal sources, high stocking densities and productivity are still possible because of the food available in natural habitats. Finding a locally accessible alternative protein source to replace the current commercial feed has become crucial due to the high demand and restricted supply of fish meals (Rani & Kumar 2020). The fish feed contains both plant- and animal-based ingredients. As a source of animal protein, fish meal, byproducts from fishing, and byproducts from terrestrial animals are all used. The fish diet contains plant proteins from soy, mustard oil cake, sunflower, rapeseed, cotton, and soon, as well (Montoya-Camacho et al. 2019). To lower production costs, maintain sustainability, and reduce the exploitation limit of fisheries, many alternative sources of protein are used in fish feed (Turchini et al. 2009, Hardy 2010). The most widely used ingredients in fish feed are fishmeal, soybean meal, groundnut oil cake etc. in developing countries are costly for farmers causing hardship for gaining due profit from aquaculture (Fasakin et al. 1999). A different protein source that has been used are fish meal, soyabean and mustard oil cake.

1.4 Objectives of the study

1.4.1 General objective

Proximate analysis in fish *Cirrihinus mrigala* by feeding different protein source diets.

1.4.2 Specific objective

i. To carry out proximate analysis of macronutrients in fry of *Cirrihinus mrigala* by feeding different protein source treatments diet.

ii. To compare the difference in proximate analysis of macro-nutrients in fry of *Cirrihinus mrigala* by feeding different protein source diets.

1.5 Rational of the study

In the field of research in fisheries, proximate analysis in fish fry *Cirrihinus mrigala* by feeding different protein source diets is still unexplored. Different Protein source diets in feed is important aspect in fish diets for growth and proximate analysis. So, the investigation on proximate analysis by feeding different locally available protein source diets in fish, will provide some valuable information about growth and proximate analysis in the fish.

1.6 Limitation of the study

Every research study faces some problems. So, this research also faces some challenges

i) The study was conducted only in fry.

ii) The study was conducted for short period.

2. LITERATURE REVIEW

Fish requires a high-quality protein for better growth and maintenance as compared to terrestrial animals (Wee & NG 1986). Principal composition in fish are 66 % − 81 % water, 16 % − 21 % protein, 1.2 % − 1.5 % mineral, 0.2 % − 25 % fat and 0 % − 0.5% carbohydrate (Ahmed et al. 2022). According to Begum et al. (2012), 96 - 98 % of fish body elements are moisture, protein, fat and ash. The knowledge of the biochemical components of fish is essential for the production of fish feed, animal feed, the fish industry, human health, nutritionists, pharmaceuticals, chemists, etc. (Shabir et al. 2018). In fish diets, the effectiveness of a variety of alternative protein sources as a partial or complete dietary replacement for fish meal has been assessed. These sources include rapeseed meal, chicken by-product meal, poultry offal meal, and single-cell protein among others (Hasan et al. 1997). In several carnivorous, omnivorous, and herbivorous fish species, it has been possible to replace a significant percentage of dietary FM or the entire diet with a proper combination of plant protein. Studying how varied combinations of plant-based foods affect fish species' growth, nutrient use, digestive physiology, and overall health is crucial (Pradhan et al. 2021). Hence, it is important to study about body proximate composition of fish prior to its consumption (Fawole et al. 2007).

According to Robaina et al. (1997), corn gluten (CGM) and meat and bone meals (MBM) in the diets of *Sparas aurata* to substitute fish meal in the feed, there was no significance difference in fish proximate analysis at the end of the experiment.

Similarly Rj & AR (2018), stated about proximate analysis and nutritive value in *Clarias gariepinus* fed with different locally available feed *Telferia occidentales*, *Moringa olefera* and *coppens* in catfish show difference in carcass body proximate analysis, In juvenile highest moisture and protein was observed in fish fed with *Telferia occidentales* were fat and Ash was higher in fish fed with *Moringa olefera*, whereas in adult moisture and protein was high in fish fed with *coppens*, fat and ash was high in fish fed with *Moringa olefera*.

According to Kikuchi et al. (1994), feather meal was used as a protein source in Japanese Flounder fish feed there was no marked difference in moisture, crude protein and crude ash contents among the dietary group but lipid seems to decrease as a dietary

feather meal increased. (Kikuchi 1999) experimented with the same fish fed with the diets that substitute defatted soybean meal with fish meal. In the proximate composition between the dietary groups examined, there was no appreciable variations in the amounts of moisture, crude protein, or crude lipid. In terms of ash content, small but significant changes were found.

A similar experiment was conducted by Singh et al. (2003),oilcake was used as a supplementary diet for the growth performance of *Cirrihinus mrigala* where Crude protein, fat, phosphorus and energy were significantly ($p < 0.05$) increased but moisture and ash decreased in fingerling fed on groundnut oil cake (GNOC) containing diet. A similar experiment was conducted in *Cirrihinus mrigala* by Pradhan et al. (2021) to observe the whole-body fatty acid profile and the effects of a diet that substitutes fishmeal for other foods on nutritional digestibility there was no difference in whole body composition of fish among the treatments.

According to Zaretabar et al. (2021), In the diet fish meal was replaced by barley protein concentrate plus white gluten in *Salmo trutta caspius*, no significant difference was observed in crude protein, fat and ash among the treatments.

Accordingly Wu et al. (2021), conducted an experiment to observe the effect of dietary rapeseed meal in farmed tilapia, the whole body and muscle composition that was not affected by dietary rapeseed meal ($P > 0.05$) but the crude lipid content of the whole body and muscle increased with increasing dietary rapeseed meal level.

Similarly Ljubojević et al. (2015), conducted an experiment to observe the effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio* L.). There was no significant difference in crude protein and ash but the group having diets with lower protein content gives significantly low moisture content.

According to NG et al. (2001), mealworm was used as a protein source in the feed of African catfish, three different diets was formulated commercial catfish pallet, mealworm and commercial pallet and mealworm only diet. Catfish fed with mealworm (solely or combination) showed significant higher body lipid, fish fed with the commercial diet showed higher moisture and ash, crude protein content did not vary between the treatments. According to Djissou et al. (2016), fish meal was replaced by

animal protein in the diet of *Clarias gariepinus*, lipid content was higher in the body of fish fed with earthworm and maggot meals based diets than in other fed and the highest protein was seen in fish that was fed with earthworm meal based diet.

According to Kim et al. (2018), tuna by-product was used as a dietary protein source replacing fishmeal in juvenile Korean rockfish; there was no significant difference in moisture, crude protein and ash contents among different experiment diets and at the same time crude lipid content was significantly ($P < 0.05$) influenced by experimental diet.

Similarly Liu et al. (2021), conducted an experiment where a fish meal was replaced by soyabean meal in juvenile redlip mullet (*Liza haematocheila*) diet there was no significant difference ($P > 0.05$) in proximate composition of their whole body and dorsal muscle protein among all treatments. The fish fed with a fish meal diet has significantly higher lipid content than other treatments ($P < 0.05$).

An experiment was conducted by Burr et al. (2013), where canola protein concentrates was used as a replacement for fishmeal and poultry by-product meal in a commercial diet for Atlantic Salmon (*Salmo salar*), the proximate composition of fish lipid, moisture or ash concentration did not vary among the treatments. Significantly, lower crude protein concentration was observed in the fish fed with the control diet compared to fish fed with the canola protein concentration diet.

According to Silva et al. (2015), body composition is related to fish size and is used to select higher protein content at a specific size for human consumption; moisture and ash content decreased linearly with the increased body weight and also body fat content increased linearly as the function of body weight.

According to Olvera- Novoa et al. (2002) conducted an experiment in tilapia (*Oreochromis mossambicus* Peters) fry diets using torula yeast (*Candida utilis*) as a source of protein, proximate composition of body dietary intake did not appear to have a significant impact on carcass composition. Similarly, Masiha et al. (2013), experimented on *Oncorhynchus mykiss* to observe the effect of dietary flaxseed oil level on the growth performance and fatty acid composition of fingerlings, there was no significant difference in moisture, protein and ash fish fed with the experimental diets were observed but the percent of lipid content was highest in fish fed with flaxseed oil

diet which was significantly ($P < 0.05$) higher than lipid content of fish fed on fish and flaxseed oil diet.

According to S & A (2013), different plant and animal protein source meal was used to fed goldfish (*Carassius auratus),* and no significant difference ($P > 0.05$) was observed in crude lipid among the group and high moisture content was in fish fed with only plant source, protein content was high in fish fed with an animal protein source and ash content was same in animal source and plant source feed but lower in an animal plant source.

Similarly Khan et al. (2003), different oilseed meal was used as a feed in *Labeo rohita* diet there was no significant difference in protein and moisture content among different dietary groups but ash and fat content were low in soyabean meal diets. As concluded by (Jahan et al. 2013), soyabean and mustard oil cake was used as feed in *Labeo rohita* fingerling, no significant difference was observed in the body composition of fingerling fed with formulated diets. Similarly (Mohanta et al. 2016), conducted an experiment by using earthworm as a dietary protein source for rohu (*Labeo rohita),* there was no variation ($P > 0.05$) in moisture and energy content among three different diets and body protein was significantly higher ($P < 0.05$)for pelleted earthworm diet groups, lipid and total ash content seems to be significantly lower ($P < 0.05$) in earthworm formulated diet than other formulated diet. Nutritional Analysis of Soybean Meal for Carp and Rainbow Trout by (Pongmaneerat & Watanabe 1993), the fish used for experimentation has a variety of bodily parts. The body protein content of either rainbow trout or carp was not significantly impacted by rising dietary protein levels. Inversely reflecting the moisture content, the lipid content increased in fish fed higher fatty diets.

According to Nandeesha et al. (1998), *Spirulina platensis* was used as a diet in Common carp to observe growth and body composition, no significant difference was observed in moisture and protein but whole lipid content is low and ash content shows some significant difference after feeding different treatments diets. As reported by (Kim et al. 2016), the different dietary level of protein was used as a diet to fish *Oplegnathus fasciatus*, protein and lipid content shows significant difference where there was no significant difference in moisture and ash content. A similar, experiment was conducted in the same species by (Kim et al. 2013) by substituting fishmeal with *Spirulina pacifica.*

Fish meal was substituted by poultry by-product meal on gilthead seabream (*Sparus aurata*), crude lipid and energy contents of fish muscle tissue and the entire body were significantly reduced by the complete replacement of FM protein by PBM. Additionally, nutrition had no impact on the moisture and crude protein contents of muscle and the entire body (Karapanagiotidis et al. 2019).

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3. MATERIALS AND METHODS

3.1 Materials used in sample preparation

i) Weighing machine
ii) Gloves
iii) Scissors
iv) Dissecting box
v) Scale
vi) Ziplock bag
vii) Aluminium foil
viii) Tissue papers
ix) Trays

3.2 Instruments used during analysis of proximate composition

i) Analytical balance
ii) Hot air oven
iii) Micro kjeldahl's digester and distiller
iv) Digestion and distillation tube
v) Muffle furnace
vi) Soxhlet apparatus

3.3 Chemical reagent used for proximate analysis

i) Sodium hydroxide **(**NaOH)
ii) Catalyst: Sodium sulphate ($Na_2\ SO_4$) and Copper sulphate ($CuSO_4$)
iii) Concentrated Sulphuric acid($H_2\ SO_4$)
iv) Boric acid ($H_3\ BO_3$)
v) Methyl red indicator
vi) Petroleum benzene ether

3.4 Methods

The feeding trial was conducted in the pond at Central Fisheries Promotion and Conservation Centre (CFPCC), Machapokhari Kathmandu for 90 days from October 2021 to January 2022.

3.5 Experimental setup

12 hapas was set in the pond (5x1x1) m^3.The experiment was conducted in a completely randomized block design under four treatments each of which treatments were replicated for three times. 50 fry *of Cirrihinus mrigala* of average length and weight 5.06 ± 0.5 cm and 1.5 ± 0.25 gm was kept in each hapa for experiment and the stock density was $10m/s^2$. Treatments 1 fish was fed with commercial feed, treatments 2 fish were fed with soyabean, treatments 3 fish were fed with fish meal and treatments 4 was fed with fish meal.

3.6 Experimental diets

Commercial feed was used as a control diet and the other three diets were formulated in Machapokhari. Different protein source soyabean, mustard oil cake and a fish meal were used to formulate those diets. Along with different protein source, wheat flour and rice bran were also used during formulation. The formulated diets and commercial feed contain 28% crude protein. Dry ingredients are used for feed formulation. Fish in treatments were fed with different formulated diets. The fish was fed twice a day. The proximate analysis of prepared feed samples in dry basis is in the table:

Table 1: The proximate analysis of prepared feed samples in dry basis

Feed ingredients	Moisture (%)	Lipid (%)	Ash (%)	Protein (%)
Musatrd oil cake	10.00	17.23	3.54	40
Soyabean meal	11.68	13.52	6.23	45
Fish meal	10.2	16.8	5.12	65
Commercial feed	9.20	8.70	3.22	28

3.7 Sample preparation

30 fish sample from each treatment was taken out from the hapas. Length and weight were 7.28 ± 0.5 cm and 3.20 ± 0.89 gm of collected samples were recorded. Those sample fish was taken to the laboratory of CFPCC for further process. Fish was manually cleaned, degutted and their gutted weight was measured with the help of a weighing machine. Samples were placed in a tray and kept in the oven for 24 hours at 65^0 temperature for drying. After completion of the drying process, the dried sample was kept in a homogenizer mixture to make it powder form for analysis and the weight of the homogenized mixture was recorded, respectively. The homogenized powder sample was wrapped in an aluminium foil and kept in a zip lock bag and refrigerated for a certain period before analysis.

3.8 Proximate analysis

Proximate analysis of a prepared sample of fish was performed in National Animal Nutrition Research Centre (NARC) Khumaltar, Lalitpur. Proximate analysis of the fish sample was analysed by using Association of Official Analytical Chemists AOAC (2000) method. Crude protein was analysed by Micro-Kjeldahl's method, for lipid ether extraction method, ash was analysed in muffle furnace and moisture by hot air oven drying method.

3.8.1 Determination of moisture

A representative clean dry sample of approximately 5 gm was weighed in an aluminium tray. The weighed sample was dried in a hot air oven at 100^0 c for 24 hours until a constant weight was obtained. The dried sample was quickly weighed after cooling at room temperature and kept in a desiccator to avoid the absorption of moisture. The difference in the weight is calculated.

$$\text{Moisture } (\%) = \frac{Initial\ sample\ weight - Final\ sample\ weight}{Sample\ weight} X\ 100$$

3.8.2 Determination of crude protein

Crude protein was determined by three different processes digestion, distillation and titration. 0.5-1 gm sample was weighed and kept in digestion tube, and about 5 gm of digestion mixture (Na_2SO_4 and $CuSO_4$ in ratio 4:1) was added to the digestion tube and

20 ml of concentrated Sulphuric acid was added very carefully to the tube, then the tube was fixed in a digestion chamber and heated for 2 hours at 400ºC and cooled. After heating the solution turn green. 100 ml of volume (Sample + distill water) in a volumetric flask was prepared and the colour of the solution turned in blue after the addition of distill water and left overnight. For distillation 10ml of solution was taken in a tube and fixed in a distillation chamber for distillation process. During distillation process the sample is mixed with 10ml of 4%boric acid, 10ml sodium hydroxide and 50ml of distilled water is mixed completely and distilled for 3 minutes. The distilled turn from dark blue to light blue. Distillate was titrated with 0.03N H_2 SO_4 until turns from light blue to transparent.

$$\text{Crude protein (\%)} = \frac{(14\ X\ Normality)\ X\ (Reading\ point\ -\ blank\ point\ x\ 6.25)}{dry\ matter\ X\ Sample\ weight}$$

3.8.3 Determination of Ash

Ash content of the sample 0.5-1 gm was determined by weighing the crucible containing the dried sample and transferring it to a muffle furnace at 550^0 c for 3 hours. The crucible containing the sample was reweighed and the difference between sample weights indicate the ash content.

$$\text{Ash (\%)} = \frac{Weight\ of\ ash}{Weight\ of\ sample} X\ 100$$

3.8.4 Determination of Fat

2 gm of the dry sample was weighed and placed in filter paper. Filter paper with a sample is placed in the thimble and the thimble was kept in the soxhlet flask. 150ml of petroleum benzene ether was poured into the flask (half of the flask) and the flask was fixed in the heating system. The solution was heated at 160^0 c for 60 minutes and cooled. The extract was removed and left overnight. The beaker was placed in an oven for 2 hours and cooled in a desiccator and the final weight was recorded.

$$\text{Crude fat (\%)} = \frac{(Flask + Fat\ weight) - Flask\ weight}{Sample\ weight\ X\ Dry\ matter} X\ 100$$

3.9 Statistical analysis

Data were mainly represented in mean and standard deviation. To test the difference between treatments one way ANOVA was performed. A significant difference was established $P < 0.05$. Statistical analysis was performed in Microsoft Excel 2016 and in Graph Pad Prism software (version 9).

4. RESULT

4.1 Moisture

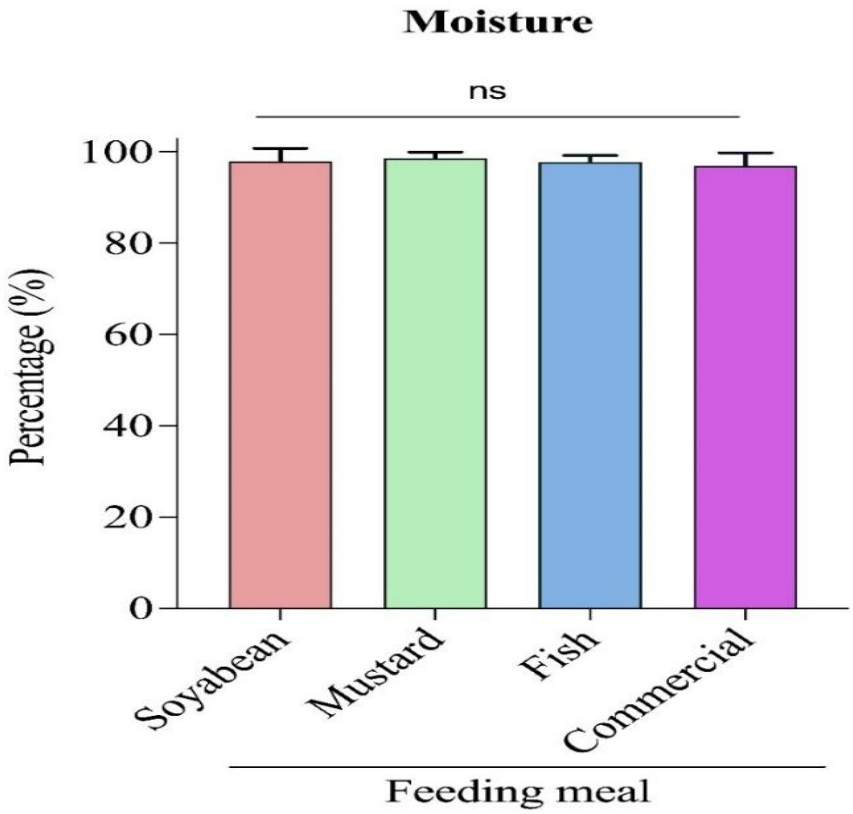

Figure 1: Moisture content in fish fed with different protein source

The moisture content in fish *Cirrihinus mrigala* fed with different protein source diets ranges from 98.56 % ± 0.59- 96.97 % ± 1.50. The highest moisture content is observed in fish fed with mustard meal and the lowest was in fish fed with commercial fish feed. Among, the group of fish there was no significant difference ($P > 0.05$) in the treatment.

4.2 Ash

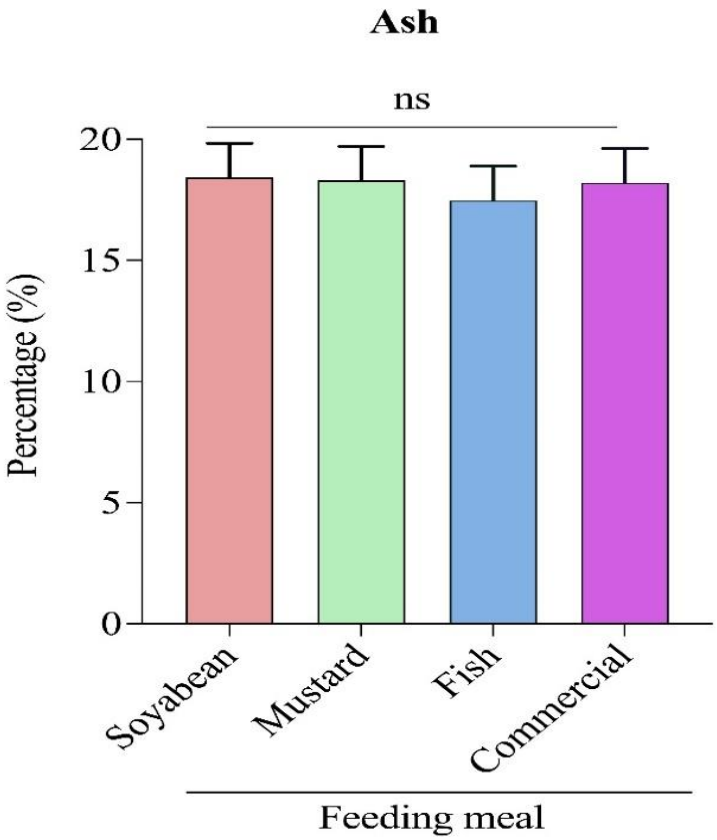

Figure 2: Ash content in the fish fed with different protein source diets.

The ash content in the fish fed with different protein source feed ranges from 18.42% ± 1.28-17.48% ± 0.89. Highest ash content was observed in fish fed with soyabean meal and lowest was observed in fish fed with fish meal. Among, the group of fish there was no significant difference ($P > 0.05$) in ash content.

4.3 Protein

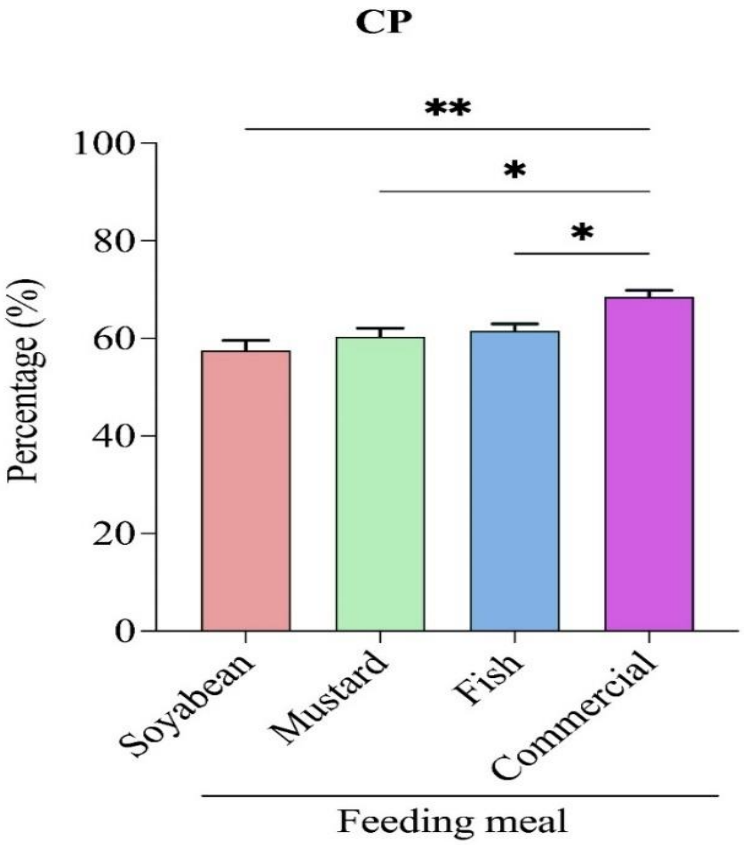

Figure 3: Protein content in fish fed with different protein source diets

The protein content in the fish *Cirrihinus mrigala* fed with different protein source diets ranges from 68.45 % ± 3.43 - 57.07 % ± 2.01. The highest protein content was observed in commercial fed fish and lowest in soyabean meal fed fish. There was significant difference in crude protein ($P < 0.05$) among the group.

4.4 Crude lipid

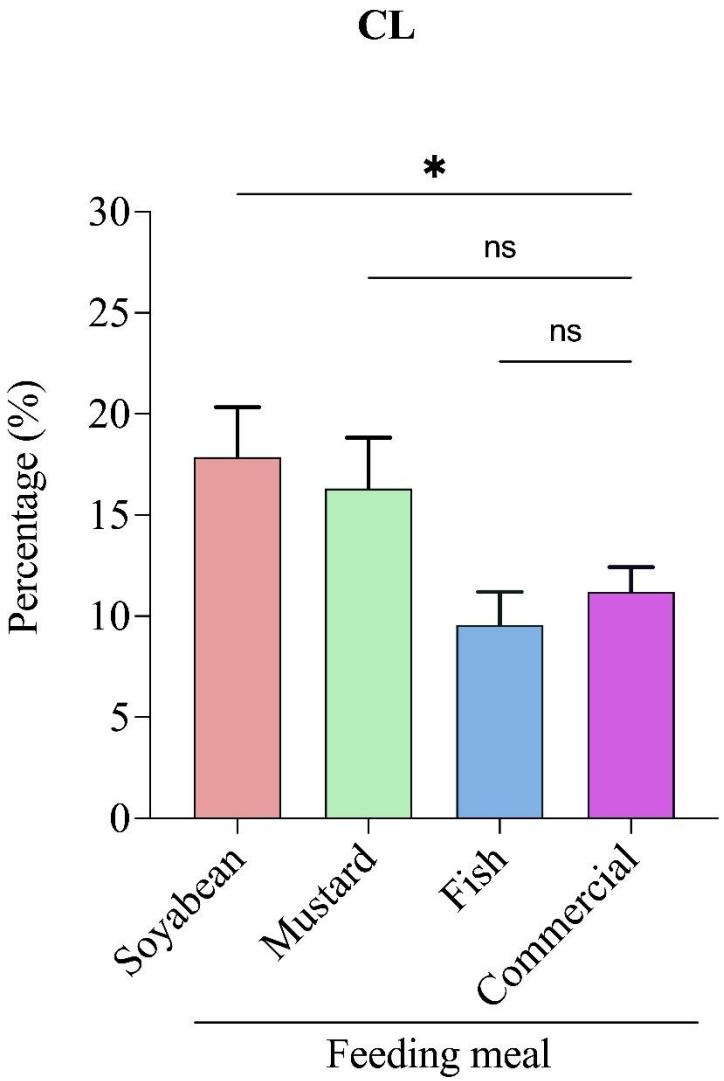

Fig: Lipid content in fish fed with different protein source diets

The lipid content in the fish *Cirrihinus mrigala* fed with different protein sources ranges from 17.88 % ± 2.46 - 9.57 % ± 1.62. The highest lipid content was observed in fish fed with soyabean meal fed fish and the lowest in fish meal fed fish. Significant difference ($P < 0.05$) was observed among the group of fish.

5. DISSCUSSION

The current study was carried out to assess proximate analysis of macronutrients in fish by feeding different protein source diets. According to Montoya-Camacho et al. (2019) different types of alternative protein source, either animal or plant origin in fish feed formulas, to reduce their production cost, maintain sustainability as well as decrease the exploitation of fisheries. Since fish feed offers both necessary and optional amino acids for the synthesis of body protein and partly supplies energy for maintenance, protein is a significant component of fish diets. Numerous studies have been conducted to establish the protein needs of fish, with estimates of protein needs based on fish species, size, dietary protein sources, and environmental factors (Yang et al. 2002). Dietary protein and energy levels affect how well food is absorbed, and these factors are mostly influenced by the diet's formulation method, nutritional makeup, and constituent quality. Growth rates, the appearance of disease, and the overall catch of fish are all negatively impacted by a lack of good-quality feed for economic production (Maldonado-García et al. 2012).

Among the parameters, moisture 98.56% and ash 18.42 % contain seem high in the whole body proximate composition. The present study strongly correlates with the finding of (Silva et al. 2015) that the moisture 67.2% and ash 3.5% content decreased linearly with the increase in body weight. The high value of ash in fish species is an indication of its high mineral content like magnesium, calcium, potassium, and zinc (BabatundeEniola et al. 2011).

The present study contradicts with the findings of (Osman et al. 2001), that low fat having fish higher moisture content was observed in mustard fed fish that is 98.56% with 16.32% high fat content and low moisture in commercial meal fed fish 96.97% with 11.20% fat content it might be due to difference in species, that is marine fish and freshwater fish.

As reported by Jahan et al. (2013), that lipid content was high in the fish fed with soyabean meal diet this finding correlates with the present study finding were lipid content was high in soyabean meal fed fish it may be due to converted carbohydrate was not utilized to provide energy. Jena et al. (1999) also concluded that lipid and protein content is high in the soyabean based diet. Lipid is an important source of

metabolic energy in fish that take part in protein sparing mechanisms (Umer et al. 2022).

Proximate composition in *Cirrihinus mrigala* Jose et al. (2006), by feeding soyabean meal diet didn't gave significant difference in moisture and fat content, while protein and ash level differed significantly among the group, this some-how contradict with present findings that no significant difference was observed in moisture and ash but there was a significant difference in protein and fat in fish.

According to the findings of Khan et al. (2003), *Labeo rohita* fed a diet containing different oil seed meals with or without a fish meal, there was no discernible difference ($P > 0.05$) in crude protein and moisture content but fish fed with Soyabean meal diet demonstrated low fat and ash content which contradict with present findings it might be an improper balance of necessary nutrients in the formulated diets. Jahan et al. (2012), there is no significant difference in proximate composition of *Labeo rohita* by feeding different experimental diets which contradicts with present finding where protein and lipid content have significant differences it might be due to the addition of high level plant protein source.

The present study contradicts with the findings of Liu et al. (2021), no significant difference was observed in whole body composition but the lipid content was high in the fish fed with fish meal diet were in present study the lipid content was high in soyabean fed meal rather then in fish meal feed it might be due to the feeding nature of the fish.

The present study support with the findings of Singh et al. (2003) and Ray & Das (1992), that oilcake as a dietary protein source in the fish feed , whole body composition was affected which also correlate with the growth pattern of the fish.

Different plant protein source treatment diets was fed to rainbow trout, no significant difference was observed in whole body proximate composition among the treatments El-Saidy & Gaber (2003) on Nile tilapia and Pongmaneerat & Watanabe(1993) which contradicts with protein and lipid content finding in present study it may be dietary protein level may affect the body composition of the fish.

Feeding different locally available feed show a significant difference in body composition in fish it's due to the composition of feed, consumption behavior and how

they utilize the feed for their metabolic activity as reported by Rj & AR (2018) in *Clarias gariepinus* this finding strongly correlate with the present study. As reported by Naeem et al. (2016), that there is a strong correlation of the body composition with the body weight , size and condition factor where the fish is being raised , this findings strongly correlate with the present finding because the surrounding where the fish being reared also affect the feeding habit, weight, size and condition factor of the fish.

According to Mohanta et al. (2007), different oil cakes like soyabean, mustard, groundnut and sesame oilcake was used in the diet of *Puntius gonionotus* fingerling and proximate composition of the whole body was higher in groundnut and soyabean diet fed fish it's due to the proper digestibility of nutrients present in diets which correlate with the present study where the growth and body composition was higher in the fish fed with soyabean meal feed than other formulated diets.

The findings of the present study correlate with the findings given by Kim et al. (2016), where lipid and protein content shows significant difference and ash and moisture content didn't show significant difference.

As concluded by Wu et al. (2021), there was no significant difference in moisture, protein and ash content but lipid content shows significant difference which correlates with the present study where lipid content shows significant difference. The difference may be due to different fish species, protein level and feed formulation.

The finding of present study correlates with the finding of Kim et al. (2013) it's due to the effects of protein source on lipid and protein levels in the body are related to both the rate of synthesis and accumulation of these substances in muscle and the organism's pace of growth.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

As, the result demonstrate that there is significant difference in lipid and protein content but not in ash and moisture in proximate analysis of fry. Therefore, soyabean and mustard meal can be used as a sole protein source instead of commercial fish feed. As, we can see protein content in fish fed with mustard give similar composition when compare to commercial fish meal. Lipid content is high in the treatments fed with soyabean and mustard meal in comparison to commercial meal. In contest of cost effective, both soyabean and mustard meal are plant derivatives and are locally available whereas commercial meal and fish meal are expensive, limited supply and have to depend on others. Soyabean meal and mustard meal are very cost effective that reduce the cost of the feed and increase the growth of fish. The study was carried out only in fry, so for more better research the same experiment must be done in adult by feeding other different source of protein. Hence, an overall study has helped to gain an idea about the proximate composition in *Cirrihinus mrigala* by feeding different protein source diets.

6.2 Recommendations

- Encouraging farmers and aqua-culturists to utilize locally available plant and animal protein sources as feed to observe fish growth.
- Proper training for farmers regarding the nutrient requirement of *Cirrihinus mrigala* is important in the formulation of diets by using local ingredients to obtain maximum growth.

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APPENDICES

Appendix-1

Proximate analysis of fish feeding different protein source diets.Value are presented in mean ± SD.

Name of feed	Moisture(%)	Ash(%)	Crude protein(%)	Crude lipid(%)
Soyabean meal fed fish	97.99±1.34	18.42±1.28	57.07±2.01	17.88±2.46
Mustard meal fed fish	98.56±0.59	18.3±1.12	60.13±2.10	16.32±2.50
Fish meal fed fish	97.83±1.51	17.48±0.89	61.53±3.05	9.57±1.62
Commercial meal fed fish	96.97±1.50	18.21±0.78	68.45±3.43	11.2±1.22

Appendix-2

Cost price of different protein source meal used in fish feed.

S.NO	**Name of ingredients**	**Cost / kg**
1	Commercial meal	RS.90
2	Soyabean meal	RS 60
3	Mustard meal	Rs 30
4	Fish meal	Rs 203

PHOTOPLATES

Experiment setup

Collection of Fishes

Measuring the length

Degutting the fish

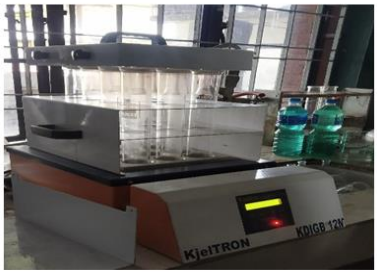
Dried fish

Homogenized sample

Prepared sample

Muffle furnace

Distillation flask



Digestion Chamber

Soxhlet apparatus

Laboratory work