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EVALUATION OF PROXIMATE AND PROTEIN CONTENT IN WALLAGO ATTU

Dhondge Sumeet[1*], Kamble Anuja Vaijanath [2] Ade Poojatai[3]

[1]Research Scholar (PhD Student), Department of Zoology, Research Center: Shri Shivaji College, Vasmat Road, Parbhani, Maharashthra*
[2]Research Scholar (PhD Student),Department of Zoology, Shri Shivaji College, Vasmat Road, Parbhani, Maharashthra
3 Research Scholar (PhD Student,Department of Zoology, Research Center: Shri Shivaji College, Vasmat Road, Parbhani, Maharashthra

ABSTRACT
Introduction: *In the world, India is amongst the top three producers of fresh water fish and shrimp fishes are rich in protein, vitamins polyunsaturated fatty acids and mineral salts and are also known as valuable protective food.*
Aim of the study: *the main aim of the study is Research on the Ayeyarwady River's Wallago attu's Nutritional Profile*
Material and method: *The Wallago attu specimens came from the city of Mandalay's Ayeyarwady River. In order to eliminate any dirt or other contaminants from the skin water was utilized to rinse and wash the samples.*
Conclusion: *Samples of Wallago attu (Nga-but) were taken from the Ayeyarwady River in the Mandalay Area. Nutritional information for Wallago attu has been calculated. However, there were a few notable outliers, such as the fact that Wallago attu's protein content was rather constant (16.1-16.4%) during the study period.*

Keywords: *Animal, Product, Protective, Wallago attu*

1. INTRODUCTION

In the world, India is amongst the top three producers of fresh water fish and shrimp fishes are rich in protein, vitamins polyunsaturated fatty acids and mineral salts and are also known as valuable protective food. Fish are the important component in our diet and rich source of protein fishes contains good quality balanced and digestible protein in fishes two types of muscle (red) and white muscles. Dark muscles contain low level of moisture and protein then the white muscle.

Pakistan, India, Sri Lanka, Nepal, Bangladesh, Burma, Thailand, Vietnam, Kampuchea, the Malay Peninsula, Afghanistan, Sumatra, and Java are all home to the giant freshwater catfish known as Wallago attu. Iots flesh's quick development and great nutritional value encourage research into the aquaculture potential of this delicious meal. A lengthy chain of amino acids joined by peptide bonds makes up the complex chemical molecules known as fish proteins. In this experiment, we need to determine how much protein is present in our sample or test the concentration or amount of protein in fish muscle sample. Fresh fish meat is a good source of protein for human diet, with humans assimilating approximately 90% to 95% of fish protein.

Based on solubility in salt solution proteins are of three groups sarcoplasmic protein, myofiibrillar protein and stroma sarcoplasmic protein (Albumin and Globulin) constitutes 25-30% of protein, myofibrillar protein (myosin, actin, tropomyosin, troponin) are structural protein constitute 65-70% of total protein stroma proteins are also known as connective tissue protein. It constitutes 3% of total protein.

What are the Essential amino acids?

The organic compounds known as amino acids are made up of nitrogen, carbon, hydrogen, and oxygen, as well as a variable side chain group. Only nine of the twenty amino acids that your body needs to grow and function correctly are considered essential, even though all twenty are vital to our health. These include tryptophan, valin, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, and threonine.

The sources of essential amino acids are animal protein like meat, eggs and poultry. When we eat protein, it broken down into amino acids which are then used to help your body with various processes such as building muscle and regulating immune function. There are several nonessential amino acids that are classified as conditionally essential. These are essential only under specific circumstances such as illness or stress. For example, although Arginine is considered non-essential your body can't meet demands when fighting certain diseases like cancer. That why Arginine must be supplemented through diet and must be obtained through your diet circumstances like

Importance of protein amino acid in human

The nine essential amino acids perform a number of important and varied jobs in your body: -

1. **phenylalanine:** Phenylalanine is a precursor for the neurotransmitter's tyrosine, epinephrine, and norepinephrine. It plays an integral role in the structure and function of proteins and enzymes and the production of other amino acids
2. **Valine:** Valien is the one of three branched chain amino acids meanings it has a chain branching off to one side of its molecular structure. Valine helps stimulate muscle growth and regeneration and is involved in energy production
3. **Threonine:** Threonine is a principal part of structural proteins such as collagen and elastin which are important components of the skin and connective tissue. It also plays a role in fat metabolism acan'tnd immune function.
4. **Trytophan:** Though often associated with causing drowsiness, tryptophan has many other functions its needed to maintain proper nitrogen balance and is a precursor to serotonin a neurotransmitter that regulates your appetite, sleep, and mood.
5. **Isoleucine:** The last of the three branched chain amino acids, isoleucine is involved in muscle metabolism and is heavily concentrated in muscle tissue. It also important for immune function, haemoglobin production and energy regulation
6. **Lysine:** lysine plays major roles in protein synthesis hormones and enzymes production and the absorption of calcium. It's also important for energy production, immuno function and the production of collagen and elastin
7. **Methionine:** Methionine plays an important role in metabolism and detoxification. It's also necessary for tissue growth and the absorption of zinc and selenium, minerals that are vital to your health.
8. **Histidine:** Histidine is used to produce histamine a neruro transmitter that is vital to immune response, digestion, sexual function, and sleep woke cycles. Its critical for maintaining the myelin sheath, a protective barrier that surrounds your nerve cells.

Essential amino acids are at the core of many vital processes through amino acids are most recognized for their role in muscle development and repair, the body depends on them for so much more. That's why essential amino acids deficiencies can negatively impact your body including your nervous reproductive, immune, and digestive systems. All nine essential amino acids perform varied roles in your body. They are involved in important process such as tissue growth, energy production, immune function, and nutrient absorption.

Importance of proteins in human diets

The human body needs proteins as a nutrition. They can be used as a fuel source in addition to being one of the components of bodily tissue. Compared to lipids, which have an energy density of g kal (37 KI) per gram, proteins have an energy density of 4Kcal (17 KI) per gram, which is equivalent to that of carbohydrates. From a nutritional perspective, the most significant feature of protein is its amino acids, which the body is unable to biosynthesize.

To avoid protein energy deficiency and consequent death, humans need to consume nine essential amino acids through their food. These include leucine, isoleucine, tysine, histidine, phenyalanone, valine, threonine, taryptophan, and methionine. The synthesis of six conditionally necessary amino acids may be restricted.

2. LITERATURE REVIEW

Mandal, Mrinmay & Das Chatterjee (2021) Many of West Bengal's original habitats for freshwater fish have seen a dramatic reduction in fish variety in recent years. Many species of freshwater fish have been removed from the Keleghai River, and many of these species are now endangered or vulnerable. The extinction of ecologically sensitive species has been linked to the collapse or crises of aquatic ecosystems caused by human activity. Boal (Wallago attu) fish were formerly numerous in this river, but nowadays it's quite a rare event to see one or reel one in. In the last several decades, there has been a dramatic decline in the population of this species. This study uses an empirical approach to research the causes of this phenomenon. Due to a dearth of written materials, our study relied heavily on the insights of seasoned fishermen to fill in the gaps in our understanding. The study concludes, based on qualitative research, that the fundamental reason for the disappearance of Attu fish in this river is the simplicity of the food web in this aquatic habitat. Modern illegal fishing methods, habitat destruction, and the heavy use of chemical pesticides and fertilizers in wetland areas are all major contributors.

Thella, Rufus & Dahanukar (2018) Wallago attu, also known as the Asian sheat catfish, has a high nutritional value and is in great demand, making it an important commercial species. W. attu asymptotic length (L) was calculated to be 99.75 cm, growth coefficient between 0.89 and 1.3 year-1, and total death rat yees between 2.23 and 3.36 year-1 based on captures from river systems in southern India, revealing population dynamics and exploitation levels. Each river system had an exploitation rate higher than the point at which the stock was reduced to half of its virgin biomass, indicating an urgent need for fisheries management strategies to be developed and put into action.

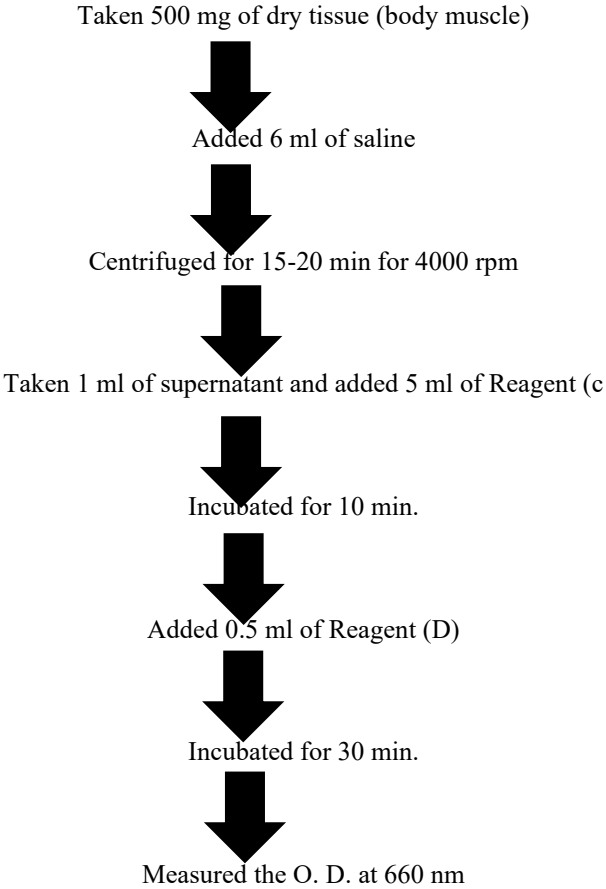
Devadawson, Chandravathany & Jayasinghe (2017) The purpose of this research was to identify highly nutritious fresh water fish and to examine the connection between fish diet and feeding behavior by measuring the total lipid content, fatty acid composition, and tocopherol content of muscle in eleven fresh water fishes from the families Cichlidae, Anabantidae, Siluridae, Cyprinidae, and Channidae that live in reservoirs in Sri Lanka. Fishes with omnivore, carnivorous, and herbivorous diets showed a wide range of muscle lipid levels, from 1.5% to 44.5%. Total lipids (41.5%), polyunsaturated fatty acids (PUFA) (39.9%), and -tocopherol (29.65mg/Kg) in the muscle were greatest in walking catfish (Clarius brachysoma) with an omnivorous eating behavior. Consumption of catfishes is advantageous to human health, and it has a very positive impact on reducing cardiovascular disorders, as shown by the walking catfish's 1 to 6 ratio of total n-3 to n-6 fatty acids. Total lipid content in the muscle of omnivores such the tilapia (Tilapia mossambica) and the climbing perch (Anabas testudineus) was 15.73 and 12.55%, and they had 5.6 and 2.5 mg/Kg -tocopherol, respectively. Total lipid in Tilapia (Tilapia niloticus) was just 1.69 percent, while tocopherol levels were only 0.08 milligrams per kilogram. The muscle tissue of the carnivorous freshwater shark (Wallago attu) has 11.29% total lipids, 4.02 mg/Kg -tocopherol, and 36% PUFA.

Waterborg, Jakob (2009) Acid hydrolysis followed by amino acid analysis is likely the most precise approach for estimating protein content. Absolute concentrations cannot be attained (1) using most other techniques because they are susceptible to the amino acid makeup of the protein. Lowry protein estimations are a perfectly acceptable alternative to a rigorous absolute determination in almost all circumstances involving protein mixtures or crude extracts, and their sensitivity is moderately constant from protein to protein. The Biuret reaction, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu+, reacts with the Folin reagent; the Folin—Ciocalteau reaction, the underlying mechanism of which is poorly understood but in essence involves the reduction of phosphomolybdotungstate to heteropoly-bdenum blue via copper-catalyzed oxidation of aromatic amino acids; and the Folin reagent. The reaction produces a deep blue hue, the intensity of which is influenced by the concentrations of tyrosine and tryptophan. The approach is most effective when used to solutions whose protein concentrations fall within the range of 0.01 to 1.0 mg/mL.

Parvez, Suhel & Pandey (2006) In order to perform the current study, water, sediment, and fish samples were taken during a fish-kill event in Panipat (Haryana, India), and then again two months later. There was no fish death on the second sample, and the water was much clearer. Lipid peroxidation (LPO) and antioxidant levels in fish tissues were investigated. Heavy metal and pesticide analysis in water and sediment samples were also performed both during and after the event, in addition to physico-chemical examination of water samples. During the fish death, the dissolved oxygen level dropped significantly. Water and sediment samples collected during the event revealed the presence of heavy metals (copper and chromium) and pesticides (BHC (Benzene hexachloride), DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane). The Indian freshwater fish Wallago attu (Bl. and Sch.) was tested for several oxidative stress indicators in its liver, kidneys, and gills. Liver samples taken from Panipat after the fish-kill showed considerably (P0.001) increased levels of reduced glutathione and non-protein thiol. There was no discernible change in ascorbic acid levels across the board following the incident. After the fish-kill event, all fish tissues were found to have LPO levels that were considerably lower than normal (P0.01-0.001). All the fish tissues analyzed showed substantially reduced protein carbonyl levels (P0.05-0.01). According to the results, commercial goods might be lost in large quantities due to industrial wastewater. The responses of the biomarkers were well-correlated with the ambient chemical stress conditions as measured by the simultaneous analysis of the physicochemical properties of the water samples.

3. METHODOLOGY

3.1 Flow Chart Showing the Extraction of Protein from Fish Body Muscles



Estimation of protein by lowerys method

i) Reagents and Chemicals

- Reagent (A): 2% Sodium carbonate (Na2CO3.5H2O) in 0.1 N sodium hydroxide.
- Reagent (B): 0.5% Copper sulphate CuSO4.5H2O in % Potassium sodium tartrate.
- Reagent (C): Alkaline copper Solution - Mixed 50 ml of Reagent (A) and 1ml of Reagent
- (B) prior to use.
- Reagent (D): Folin - Ciocalteau Reagent.
- Protein Solution (Stock Solution): 50 mg of BSA was accurately weighed and dissolved in distilled water and made the volume up to 50 ml in a standard flask.
- Working standard-: 100 ml of the stock solution was diluted into 50 ml of distilled water in a standard flask. 1ml of this solution contains 200 ug proteins

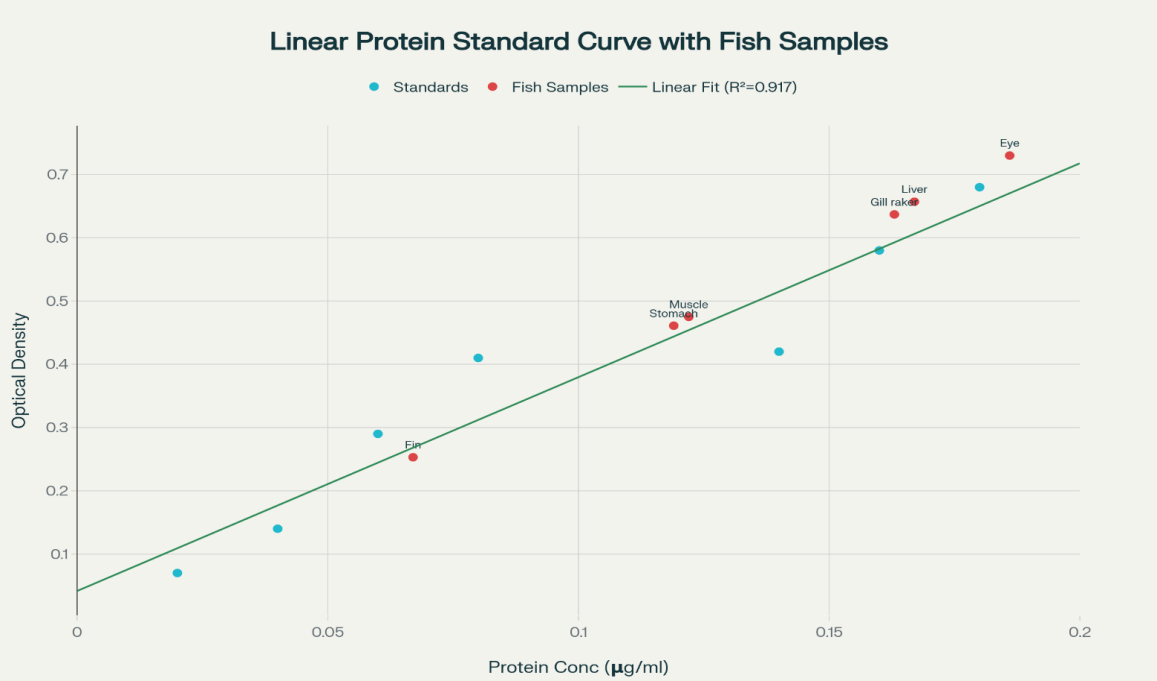
Procedure

- 0.2 ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water. The test tube with 1 ml distilled water serves as blank.
- Add 4.5 ml of Reagent I and incubate for 10 minutes.
- After incubation add 0.5 ml of reagent II and incubate for 30 minutes
- Measure the absorbance at 660 nm and plot the standard graph.

Estimate the amount of protein present in the given sample from the standard graph

Sr. no.	**working solution**	**distilled water (ml)**	**Concentration of protein (ug/ml)**	**Alkaline solution (ml)**		**Phenol reagent**		**Optical density**
1	0.1	0.9	0.02	5		0.5		0.07
2	0.2	0.8	0.04	5		0.5		0.14
3	0.3	0.7	0.06	5		0.5		0.29
4	0.4	0.6	0.08	5	**inbu.**	0.5	**Incu**	0.41
5	0.5	0.5	0.10	5	**10min**	0.5	**30min**	0.36
6	0.6	0.4	0.12	5		0.5		0.37
7	0.7	0.3	0.14	5		0.5		0.42
8	0.8	0.2	0.16	5		0.5		0.58
9	0.9	0.1	0.18	5		0.5		0.68
10	1.0	0	0.20	5		0.5		0.95
11	Blank	1.0	-	5		0.5		0.0

Gill raker	0.1	0.9	0.185	5		0.5		0.637
Stomach	0.1	0.9	0.125	5		0.5		0.461
fin	0.1	0.9	0.07	5		0.5		0.253
eye	0.1	0.9	0.200	5		0.5		0.73
muscle	0.1	0.9	0.130	5		0.5		0.475
liver	0.1	0.9	0.170	5		0.5		0.657



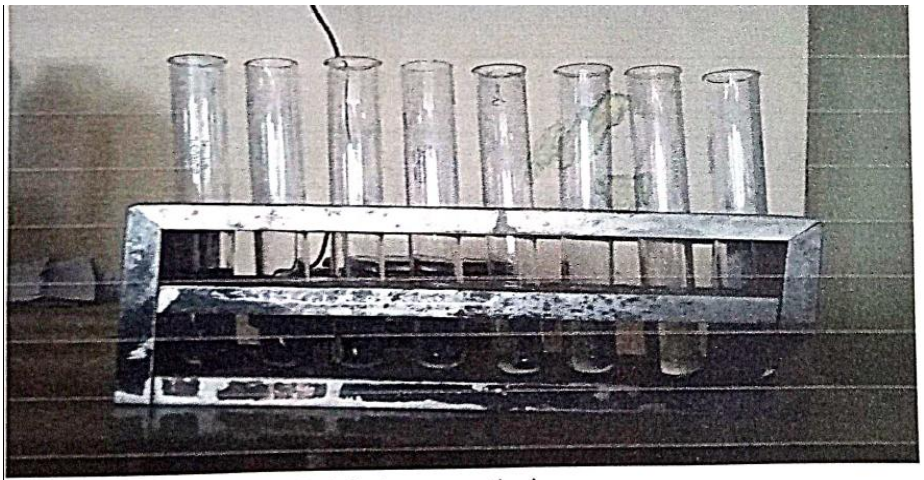


FIGURE . Folin lowery method

Sample Collection

The Wallago attu specimens came from the city of Mandalay's Ayeyarwady River. In order to eliminate any dirt or other contaminants from the skin, the samples were rinsed with tap water.

Determination of Ash Content of Wallago attu

The Wallago attu was dissected down to its marrow, muscle, bone, skin, and organs; then, around 3g of each sample was properly weighed and placed in a porcelain crucible that had been overheated, cooled, and weighed. The hot plate was used to sear them. Then they went into the muffle furnace at 600 C until the reddish-brown residue had become white all over. After being placed in the desiccator, it was finally weighed. It took many cycles of heating, cooling, and weighing to get a stable weight.

Biochemical analysis

The A. M. C. approach (1979) was then used to simultaneously estimate TVB-N and TMA-N. The remaining samples were analyzed in the lab using the A. O. A. C. (1980) technique to determine the percentages of protein, fat, ash, and moisture.

4. RESULTS

4.1 Determination of Physicochemical Properties of Wallago Attu

Nga-but's physical and chemical characteristics in muscle, fat, bone, skin, and internal organs were measured and tabulated.

Table 4.1 The ash contents of flesh, bone, skin, and internal organs of Wallago Attu

Samples	Ash contents (%)		
	1st	2st	3st
flesh	0.93	0.86	0.86
bone	3.56	3.49	3.49
skin	1.35	1.25	1.25
Internal organs	1.20	1.12	1.12

The ash percentage of Wallago attu was found to be between 0.86% and 0.93% in the flesh, 3.49% and 3.56% in the bone, 1.25% and 1.35% in the skin, and 1.12% and 1.20% in the internal organs, as shown in Table 4.1.

Table 4.2 Determination of water content of flesh of Wallago attu

No.	Water content (%)
1	55.55
2	54.77
3	54.77

This table shows that the water content of Wallago attu meat ranges from 54.77 percent to 55.55 percent.

Table 4.3 Kjeldahl's Methods Applied to the Analysis of Nitrogen and Protein in Wallago attu Flesh

No.	Nitrogen (%)	Protein (%)
1	3.21	20.06
2	3.28	20.50
3	3.28	20.50

This table shows that the nitrogen concentration of meat ranges from 3.21% to 3.28%, whereas the protein content ranges from 20.06% to 20.50%. Nutritionally, wallago attu is excellent. The human body requires protein, which may also be converted into fuel.

CONCLUSION

The physicochemical analysis of Wallago attu showing that each part of fish have different protein concentration determined using the Folin-Lowry standard curve method. The muscle, liver, and eye showed relatively higher protein levels compared to fin and stomach. The fish flesh is nutritionally excellent, containing 20.06–20.50% protein, which is essential for human health and metabolism. Additionally, significant variation in ash and water content among tissues demonstrates the diversity in composition, with bones being mineral-rich. Overall, Wallago attu is a valuable source of dietary protein and minerals, useful for nutrition studies and food science.

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