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CHAPTER 15

Amino Acid Composition in Edible Wallago Attu Muscles Represents a Significant Variation with Changes in Habitats in Different Seasons from the Godavari River Basin, India

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Abstract

This study aimed to investigate the total amino acid composition in the body muscles of *Wallago attu* in relation to the habitat difference from the Godavari River basin, India. The total amino acid content was found 11.02±1.07 to 19.80±1.70 mg/g and 10.95±1.01 to 20.57±1.31 mg/g in the body muscles of *Wallago attu* collected from all selected habitats in different seasons during Feb. 2010 - Jan. 2011 and Feb. 2011 - Jan. 2012. The total amino acid content in the body muscles of *Wallago attu* collected in the summer season from Masoli Reservoir was higher, but the total amino acid content in body muscles of *Wallago attu* collected from Siddheshwar Reservoir in the winter season was lower. The present study revealed that there exists a significant variation ($p<0.05$) in the total amino acid (mg/g) content in the *Wallago attu*'s body muscles, which was collected from all four selected habitats during distinct seasons. Therefore, Fish consumers may need to decide the purchase of these common food fish species from fish markets according to their need for amino acids.

Keywords: *Wallago attu*, Amino acid, Reservoirs, Marathwada

Introduction

Fish are an important food commodity in the human diet since ancient times. *Wallago attu* (Bloch and Schneider, 1801) is an important fish species regularly found in the catch from the Godavari river basin in the South-Central region of India. There are reports on the estimation of amino acid analysis of body muscle tissues

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of edible fishes from various selected habitats around the globe. In the present investigation, commonly found predatory freshwater teleost *Wallago attu* from the selected study area was analyzed for the amino acid compositions concerning habitat differences to determine variation in the nutritional contents with the change in habitat or locality. It was found that there was no major difference in the composition of amino acids in selected species. But with the change in their habitat like a reservoir and with the change in seasons they found significant differences in amino acid concentration. During the winter season, the amino acids in the fish species decreased while it increased during the summer season. Hence the fish consumers from this region need to think about the habitat of fish and the season of consumption to fulfill their need for amino acids.

The amino acids are essential for the formation of all proteins. Free amino acids are the amino acids that are free-form in tissues and do not bind to proteins. The ninhydrin method, introduced by Moore and Stein in 1948, is widely used to estimate the total free amino acids. Two functional groups, amino (-NH_2) and carboxyl (-COOH), make up the group of organic compounds known as amino acids. Nutritional guidelines categorize amino acids into two categories: essential amino acids or dispensable amino acids, and non-essential amino acids or indispensable amino acids. Essential amino acids are those that cannot be synthesized by the body and must be supplied through diet. They are necessary for an individual's normal growth and maintenance. The body can produce about 10 amino acids to meet its biological needs, so there's no need to eat them through diet.

Various temperature ranges lead to an increase in the level of free amino acids when exposed for different periods of time (Tantarpale et al., 2011). The healing process is mostly dependent on amino acids, and an essential amino acid deficiency may impede the recovery process (Deka et al., 2012). *Channa striatus* muscles possess a high concentration of arginine and amino acids, which contribute to wound healing process (Lay H. G. et al., 2005). Essential amino acids are required daily for healthy living, as per the FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition 2007. There was some variation in the quantity of amino acids among Tilapia species. But, values are quite near to each other. The amino acid composition differed significantly among fish species like *Oreochromis aureus*, *Tilapia rendalli*, *Tilapia spp.*, *Oreochromis niloticus*, and *Tilapia zillii* (Tasbozan et al., 2013), *Clarius gariepinus* and *Tilapia zillii* have been reported as important sources of nutrients. The use of essential amino acids were utilized to boost good health, prevent, and heal ailments in humans (Osibona et al., 2009).

Materials and Methods

Fish samples and sampling sites:

The freshwater shark *Wallago attu* (Bloch and Schneider, 1801) was collected from the rivers Dudhana, Purna, and Godavari; the fish samples were also collected from reservoirs like Karpara, Masoli, Yeldari, and Siddheshwar in Marathwada Region of Maharashtra State, India from the fishermen. Selected fish species from fresh catch were collected monthly from February 2010 to January 2011 and February 2011 to January 2012 from the selected fishing sites.

The Ninhydrin method was employed to determine the total amino acid content of the body muscles of particular fish species from various chosen environments (Moore and Stein, 1948).

Triketohydrindene hydrate, another name for ninhydrin, is a chemical compound that, when combined with amino acids, produces Riemann's purple, a color complex with a maximum absorption wavelength of 570 nm. Ammonia and carbon dioxide are released when the amino acid is oxidized to aldehyde by ninhydrin. Ninhydrin is converted to hydridatin throughout the process. When ammonia is present, the hydridatin form condensed with Ninhydrin to produce a purple hue complex.

Procedure for total amino acid extraction

1) 20 mg of sample was taken, and then phosphate buffer (pH 7.0) was added.

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2) Phosphate buffer was used to crush the sample.
3) The mixture was centrifuged for 20 minutes at 10,000 rpm.
4) After removing the supernatant, the pellet was dried.
5) After adding the pH-7.0 phosphate buffer, the pellet was suspended.

Standard stock solution for amino acids (10µl) or 1.31 mg/g.

1) Leucine was weighed at 1.31 mg. It was carefully placed in a 1000 ml volumetric flask. There was 20–30 ml of distilled water used to suspend the amino acid and heated for 10 minutes in a hot water bath. Distilled water was added to make the volume to 100 ml when it cooled.
2) In a volumetric flask, dilute 10 ml of stock solution with 100 ml of distilled water to produce the working standard solution (1 ul mole/ml or 1.31 ug/ml).

Reagents

1) *0.2 M citrate buffer at pH 5.0*

150 ml of distilled water was used to dissolve 10.51 grams of (monohydrate) citric acid. To get the pH of the solution to 5.0, NaOH solution (1N) was added, and the volume was raised to 250 ml using distilled water in a volumetric flask.

2) *Reagent for ninhydrin*

The pH of the solution was raised to 5.0 by dissolving 0.4 grams of stannous chloride (monohydrate) in 250 ml of 0.2M citric acid buffer. This mixture was added to 150 ml of methyl cellosolve ethylene glycol monomethyl ether, containing 1 g of ninhydrin, and then it was mixed. A brown bottle was used to store the reagent. The reagent for ninhydrin was made fresh for the analysis.

3) *Diluent solution: A 1:1 ratio of n-propyl alcohol to distilled water was used.*

Procedure

1) The unknown sample solution was diluted roughly and 2.0 ml of Ninhydrin reagent was added to the standard solution 0.1 ml (having 0.1–1.0 µl mole of L-leucine in it.). A hot water bath was used for fifteen minutes to heat the test tubes that had glass marbles enclosed within. 7 ml of the diluent solution was added to the tubes, which were then vortexed after cooling to room temperature.
2) In the spectrophotometer, the purple color was generated against the reagent blank at 570 nm. The absorbance was noted.
3) The leucine concentration (0.1-1.0µl) on graph paper on the x-axis and absorbance at 570 plotted on the y-axis to create a calibration curve. Using a concentration curve, amount of amino acid in the sample was calculated.

Statistical Analysis

The mean ± standard deviation obtained from the analysis of fish species is the current result for the freshwater fish *Wallago attu*. Using Microsoft Office Excel 2007, a two-way analysis of variance is applied to the mean values of the total amino acid content. A statistically significant difference is noted ($p<0.05$). A Fisher's T-test was run to identify differences between the reservoirs and seasons if the overall F-test was significant ($p<0.05$).

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Results

The outcomes of the total amount of amino acids found in *W. attu* body muscles, which were taken from all chosen habitats in various seasons between February 2010 and January 2011, and between February 2011 and January 2012, are described here.

Between February 2010 and January 2011, body muscle's total amino acid content was identified to be 11.02±1.07 to 19.80±1.70 mg/g, obtained from all selected habitats in different seasons. When body muscles were taken from Masoli Reservoir in the summertime, the highest total amount of amino acids was 19.80±1.70 mg/g observed. Table 1.1 and Figure 1.1 show that, when compared to all other selected habitats in various seasons, the lowest total amino acid concentration was found in the body muscles which were obtained from Siddheshwar Reservoir during the winter season at 11.02±1.07 mg/g. seasons as shown in Table 1.2 and Fig. 1.2.

The results of the Two-way ANOVA comparing reservoirs and seasons show that, as shown in tables 1.1 (a) and (b); 1.2 (a) and (b), there is significant variation ($p<0.05$) in the total amino acid (mg/g) content of *Wallago attu's* body muscles between February 2010 and January 2011.

Table 1.1 shows the seasonal change of the total amino acid content (mg/g) in *Wallago attu* body muscles that were obtained from chosen habitats between February 2010 and January 2011.

Seasons ↓ / Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	18.05±1.75	19.80±1.70	19.00±1.25	13.00±1.55
Monsoon	17.97±1.36	19.32±0.78	18.00±1.04	13.55±0.62
Winter	14.15±1.28	17.4±1.11	14.90±2.53	11.02±1.07

The data is shown as mean ± standard deviation (n=10)

Table 1.1 (a) - ANOVA: Two factors without replication for Total Amino acid (mg/g)

SUMMARY	Count	Sum	Average	Variance	SD
Summer season	4	4	69.85	17.4625	9.362292
Monsoon season	4	4	68.84	17.21	6.3498
Winter season	4	4	57.47	14.3675	6.910892
Karpara Reservoir	3	50.17	16.72333	4.968133	2.228931
Masoli Reservoir	3	56.52	18.84	1.6128	1.269961
Yeldari Reservoir	3	51.9	17.3	4.57	2.137756
Siddheshwar Reservoir	3	37.57	12.52333	1.770633	1.330651

Table 1.1(b) - Analyzing Variance (ANOVA) for Total Amino Acid (mg/g) is shown below.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Seasons	23.63012	2	11.81506	32.03336	0.000628	5.143253
Reservoirs	65.65593	3	21.88531	59.33614	7.49E-05	4.757063
Error	2.213017	6	0.368836			
Total	91.49907	11				

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Table 1.2 shows the seasonal change of the total amino acid content (mg/g) in *Wallago attu* body muscles that were obtained from chosen habitats between February 2011 and January 2012

Seasons ↓ Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	18.97±1.47	20.57±1.31	17.22±1.12	14.97±1.55
Monsoon	17.92±1.13	20.27±0.84	15.10±1.49	14.12±1.03
Winter	13.80±1.25	16.87±1.48	11.8±0.77	10.95±1.01

The data is shown as mean ± standard deviation (n=10).

Table 1.2 (a) - ANOVA: Two factors without replication for Total Amino acid (mg/g)

Summary	Count	Sum	Average	Variance	SD
Summer season	4	71.73	17.9325	5.772292	2.402559
Monsoon season	4	67.41	16.8525	7.785558	2.790261
Winter season	4	53.42	13.355	6.918433	2.630291
Karpara Reservoir	3	50.69	16.89667	7.467633	2.732697
Masoli Reservoir	3	57.71	19.23667	4.223333	2.055075
Yeldari Reservoir	3	44.12	14.70667	7.460133	2.731324
Siddheshwar Reservoir	3	40.04	13.34667	4.488633	2.11864

Table 1.2(b) - Analyzing Variance (ANOVA) for Total Amino Acid (mg/g) is shown below

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Seasons	45.80322	2	22.90161	93.0802	3.04E-05	5.143253
Reservoirs	59.9526	3	19.9842	81.22283	3.01E-05	4.757063
Error	1.47625	6	0.246042			
Total	107.2321	11				

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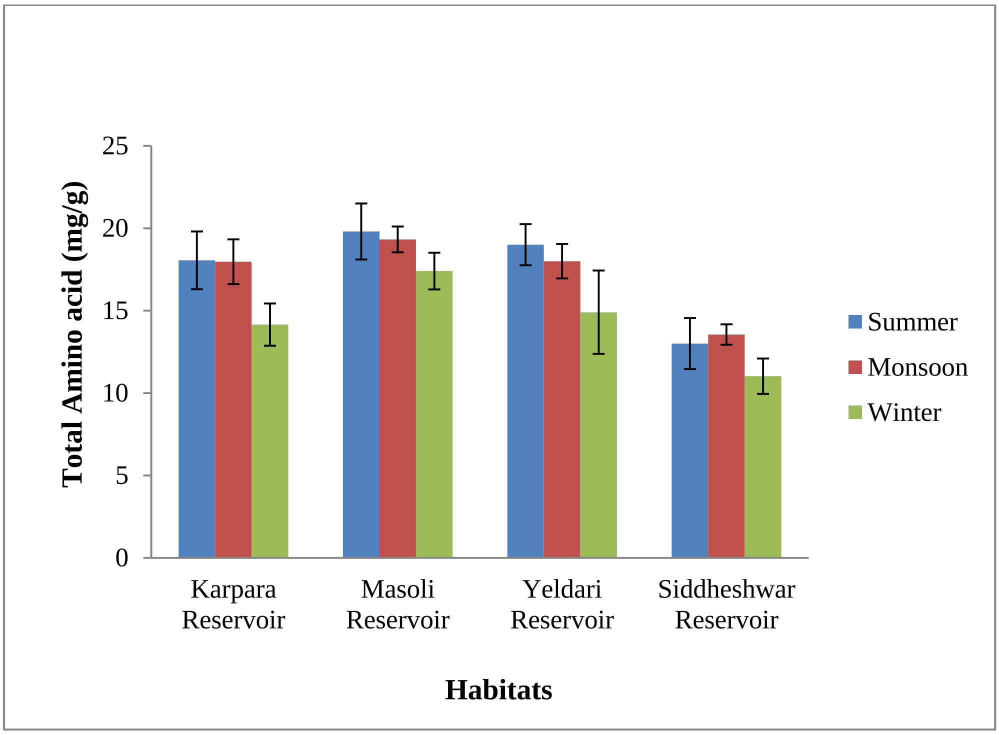

Fig. 1.1 shows the seasonal change of the total amino acid content (mg/g) in *Wallago attu* body muscles that were obtained from chosen habitats between February 2010 and January 2011.

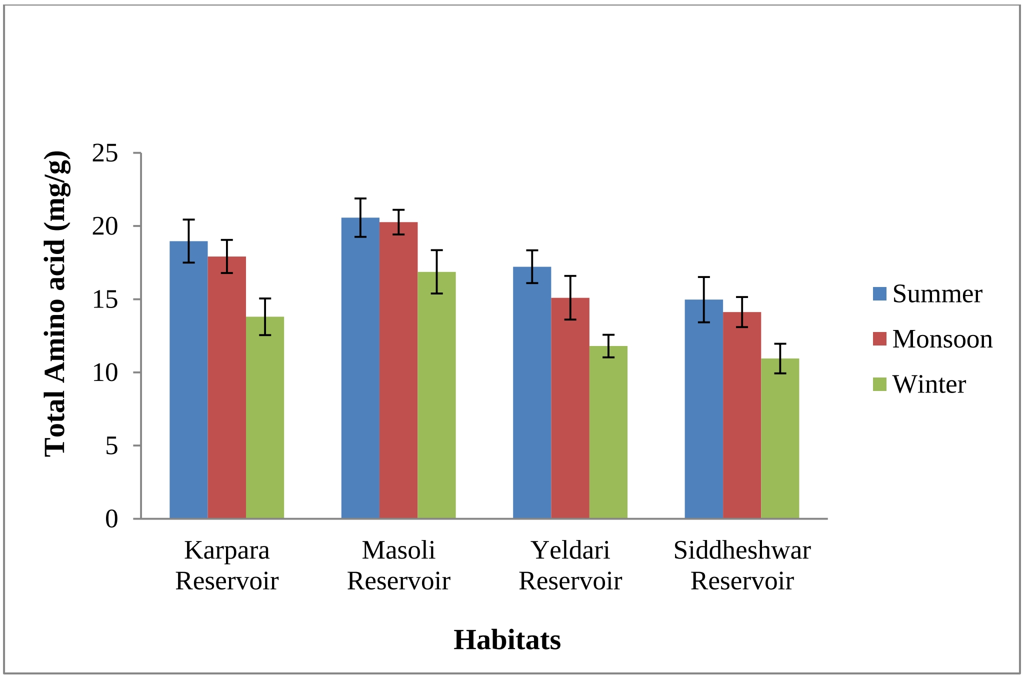

Fig. 1.2 - shows the seasonal change of the total amino acid content (mg/g) in *Wallago attu* body muscles that were obtained from chosen habitats between February 2011 and January 2012

Discussion

In *Wallago attu* body muscles obtained from several reservoirs in different seasons between February 2010 and January 2011, and February 2011 and January 2012, there is a significant ($p<0.05$) variation in total amino acid (mg/g). The complete findings are provided in tables 1.1, 1.1 (a), and (b) above; tables 1.2, 1.2 (a), and (b) are summarized as follows.

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In the fish muscle of *Channa striata*, Tantarpale et al. (2011) found that the levels of free amino acids increased during temperature fluctuations of 30–50°C and 10–20°C at varied exposure times. The free amino acid levels during the control condition were at 24 hours 2.76 mg/lit, at 48 hours 3.13 mg/lit, at 72 hours 3.18 mg/lit, and at 96 hours 2.65 mg/lit. Total essential amino acid content was found to be highest in *Tilapia zillii* (42.17 g/100 g) and the lowest (134.83 g/100g)) in Tilapia spp. in Tasbozan et al. (2013) investigation. The total amount of dispensable amino acids was observed to be highest (38.76 g/100 g) in *Tilapia zillii* and lowest (29.94 g/100 g) in *Oreochromis aureus*. whereas in the present investigation, the total amino acid content in *W. attu* was analyzed in relation to their habitat difference despite correlating the results with the parameters of water bodied, but there are clear differences in the water temperature of the selected reservoirs in summer, winter and monsoon seasons in the selected study area which is in the same zoogeographical area where in the district, the highest temperature recorded is 42°C. during the summer season and in the winter, the lowest temperature is between 5 and 6°C (Niture S. D. 2009).

According to P. Anil Kumar (2014), there was a 14.1% increase in the free amino acid content in the liver and intestine, 32.9% in *Catla catla*, and a 27.00%, 48.8% increase in *Labeo rohita*. In the present study, the total amino acid content was analyzed from the entire edible body muscle contents of the two most commonly occurring predatory fishes *W. attu.*

According to Neelima et al. (2011), under control conditions, the free amino acid value in *Cirrhinus mrigala* ranged in the muscles, from 18.72–18.75 mg of nitrogen per gram of wet weight, and in the gills, from 11.96–11.98 mg. in the muscles, and 14.52–14.54 mg in the liver.

According to Diniz et al. (2013), the muscles of *Dactylopterus volitans* (99.2 g/100 g), *Genypterus brasiliensis* (95.2 g/100 g), *Mullus argentinae* (96.6 g/100 g), *Paralichthys patagonicus* (95.7 g/100 g), *Percophis brasiliensis* (96.3 g/100 g), *Pinguipes brasilianus* (95.6 g/100 g), *Rhizoprinodon lalandii* (95.4 g/100 g), *Rhizoprinodon porosus* (99.7 g/100 g), and *Urophycis cirrata* (97.1 g/100 g) were found to have a similar level of amino acids, with large amounts of glutamic acid and lysine and low amounts of histidine. Whereas in the present investigation, the total amino acid content was analyzed (Tables 1 and 2).

The study conducted by Lay H. G. et al. (2005) examined the total amino acid content of *Channa striatus* muscles in three different-sized batches. The batches that were observed in November 2002 were B1 with 65.431 g/100g dry sample, B2 with 64.031 g/100g dry sample in January 2003, and B3 with 71.126 g/100g dry sample in April 2003. The outcome showed that during dry season, the content of fish proteins in B1 & B2 was lower than that of the fish in batch B3 during the rainy season. In the present study, the fish species were selected from commonly occurring fish body weight sizes in the range of 110 gm (20-25cm) to 1500 gm (60 - 65 cm) of *W. attu* respectively.

Hawaibam et al. (2014) reported that the highest essential and non-essential amino acids were found in *Labeo pangasia* (66.5 g/100g protein) and *Semiplotus manipurensis* (56.74 g/100g protein). The total amount of amino acid was found to be a maximum of 113.42 g/100 g protein in *Semiplotus manipurensis* and a minimum in *Neolissochilus stracheyi* (104.42 g/100g protein). Huda Elgubbi et al. (2015) noticed an improved procedure for amino acid detection on a TLC plate by employing stannous chloride ($SnCl_2$) and ninhydrin reagent for quick and simple identification. According to Osibona et al.'s (2009) analysis, *Clarius gariepinus* had a total amino acid content of 168.84 mg/g and *Tilapia zillii* of 170.26 mg/g. The Present study was conducted from the fish samples in fresh catch at the site of fishing and fish markets with fresh non-preserved and non-processed fish of the selected species.

Conclusion

The total amino acid (mg/g) content of *Wallago attu's* body muscles collected from the same habitat does not differ significantly ($p<0.05$), but the total amino acid (mg/g) content of these fish species' body muscles collected from various reservoirs in different seasons during February of 2010 to January of 2011 and from

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February of 2011 to January of 2012 does differ significantly ($p<0.05$). Hence there is a significant difference in the amino acid content in the selected fish species with change in habitat and change in season of a year. Therefore, it is significantly mattering from where these fish are captured.

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