

## Nutritional Impact on Protein Metabolism of Muscle and Liver Tissue of Different Fish Species *H. molitrix*, *C. carpio*, *C. idella*

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### ABSTRACT

*Agrimin and Fishmin are used as supplementary diets in many districts of Andhra Pradesh for effective fish culture. Since the current study is aimed at studying the effect of selected Supplementary feeds on fish productivity aspects, yield profiles and metabolic aspects of selected cultivable fishes, Hypophthalmichthys molitrix, Cyprinus carpio, tenopharyngodon idella, such as total proteins, free amino acids and the activities of ALAT/AAT/GDH were studied. Agrimin or fishmin treatment enhanced in the all metabolic aspects which were selected for the study and all the changes were found to be statistically significant over their corresponding control values. The muscle and liver of the control H.molitrix appeared to possess more proteins compared to other two species. The agrimin and fishmin fed fish species muscle and liver showed more percent elevations of their total protein content and the changes were found to be statistically significant over the control. Identical trends were also obtained for the muscle and livers free amino acid content. Agrimin or fishmin treatment enhanced the fish muscle and liver protease content and all the changes were found to be statistically significant over their corresponding control values. Liver tissue showed more AAT / ALAT/GDH levels. Agrimin and Fishmin fed fishes muscle and liver showed an increase in their AAT, ALAT and GDH activity levels. The muscle and liver tissues of the control feed fed fish batch appeared to possess higher AAT / ALAT/GDH levels compared to other species of fishes selected for the study.*

**Keywords:** Agrimin, Fishmin, AAT, ALAT and GDH

### INTRODUCTION

The word protein was coined by Jons J. Berzselius, The famous Swedish chemist in 1838 and derived from the Greek work Proteios (meaning of the first rank). Proteins include several important cell constituents such as enzymes, peptide hormones, antibodies, transport molecules and components of cell skeleton including cell wall. Proteins are the most characteristic chemical compounds found in the living cell. They have high molecular weight and each protein is composed of approximately 20 different kinds of amino acids linked to each other in large numbers. Many proteins contain all of the 20 amino acids. Proteins constitute about 1/5th of the animal body on the fresh weight basis. Protein budge of the cell can be taken as an important diagnostic tool in evaluating its physical standards<sup>1</sup>. Proteins may be hydralised to form amino acids on one hand and may be mobilized for protein synthesis on the other hand. Dietary protein plays a dominant role in promoting growth and robust health condition of fishes<sup>2</sup>. The amino acids have a great variety of chemically reactive groups, which results in a wide range of reactivity of a protein when exposed to inorganic and organic compounds.

In addition to covalent bonds, which bind amino acids to each other, proteins possess weaker but very important bonds that hold the macromolecule in a unique configuration. Such bonds are quite sensitive to environmental conditions – eg. excessive stirring of a protein solution in air, exposure to ultraviolet light, elevated temperatures, marked changes in pH, and organic solvents. These procedures lead to alteration of protein structure characterized by loss of solubility and of any biological activity, even though covalent bonds may not have been broken. The protein is said to be denatured and frequently the change is irreversible; the native state has been destroyed. Occasionally, changes in environmental conditions lead to dissociations of a protein into molecules of smaller size, or of association into larger aggregates. Chemical as well as biological properties of the protein are affected by such changes. A change in the levels of the Amino acid content is an indication of either extensive protein turnover or protein catabolism. In accordance to protein levels, a decrease in amino acid levels has been observed suggesting protein synthesized rather than degradation. In view of the primary role of the amino acids as osmoeffectors and energy precursors under altered environmental conditions, these hydrolytic products of proteins are analyzed both qualitatively and quantitatively to assess the role of individual amino acid species in osmotic and acid base balance and energy metabolism of Fingerlings under Ammonia stress<sup>3</sup>. Fish muscle contains a comparatively higher amount of amino acid in composition to their warm blooded successors. Fishes in general tend to possess greater proportions of leucine, isoleucine, and lysine in comparison to other animals. As far as amino acid composition is concerned, white muscle differs very little from the superficial dark muscle. Free amino acids generally increase in the tissues undergoing active protein synthesis. This increase is especially noticed in liver, but not in muscles. The free amino acid pool which is present in different tissues of piscine body has been speculated to play two basic vital roles viz., may assist osmoregulation in hypertonic environment and acts as a chemical signal (Olfactory stimuli) for the communication with other fishes<sup>4</sup>. Thus, protein metabolism involving its degradation and synthesis serves as one of the chief physiological events associated with the adaptive mechanisms, maintaining the homeostasis in metabolism under different environmental conditions. An attempt is made on a few aspects of protein metabolism during nutritional stress in exotic Major carp *C. carpio*, *C. idella*, *H. molitrix*.

## MATERIALS AND METHODS

### Chemicals and Supplementary Feed

Agrimin and Fishmin which are commercially available have been selected for the study. All other chemicals used are of technical grade from sigma, St. Louis, USA, SDH, CDH (India).

### Estimation of total proteins:

The total proteins in the tissues were estimated using the folin phenol reagent method as described by<sup>5</sup>. A 1% homogenate (W/V) was prepared in ice-cold 0.25 M sucrose solution. For soluble and structural proteins, 1.0 ml of the homogenate was taken and centrifuged at 3000 rpm for 10 minutes. The supernatant was separated and 3 ml of 10% trichloroacetic acids was added to both the supernatant and residue and again centrifuged at 3000 rpm. The supernatant and the residue were taken for experimentation. All the three residues were dissolved in 5 ml of 0.1 N sodium hydroxide and to 1 ml of each of these solutions 4 ml of reagent – D (mixture of 2% sodium carbonate and 0.5% copper sulphate in 50:1 ratio), was added. The samples were allowed to stay for 10 minutes, at the end of which 0.4 ml of folin phenol reagent (diluted with distilled water in 1:1 ratio before use) was added. Finally, the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 600 nm. A mixture of 4 ml of reagent-D and 0.4 ml of folin phenol reagent was used as blank. Bovine albumin was used for the preparation of protein standards. The protein content is expressed as mg/g wet wt of the organ.

### Estimation of free amino acids:

Free amino acid levels in the tissues were estimated by the ninhydrin method as described by Moore, S. and Stein, W.H. A<sup>6</sup>.

5% organ homogenates (W/V) were prepared in 10% trichloroacetic acid and centrifuged at 2000 rpm for 15 minutes. To 0.2 ml of supernatant, 2.0 ml of ninhydrin reagent was added and the contents were boiled for exactly 5 minutes. They were cooled under tap water and the volume was made to 10ml with distilled water. The optical density of the colour developed was measured in a spectrophotometer at a wavelength of 570 nm. A blank using distilled water and amino acid standards were also run similarly. The free amino acid levels were expressed as mg amino acid nitrogen, released / gm. wet wt of the tissue.

#### **Assay of Protease activity:**

Protease activity was estimated by the method of Moore and Stein (1954). 5% homogenate of tissues were prepared in ice-cold distilled water and 0.5 ml was used. The homogenates were centrifuged at 1000 rpm for 15 minutes. The supernatants were employed for enzyme assay. The mixture of 2.0ml contained 100M of phosphate buffer (pH 6.8) 12 mg of heat denatured hemoglobin as substrate and 0.5ml of the homogenate supernatant. The contents were incubated at 37°C for 15 minutes. The reaction was stopped by adding 2 ml of 10% TCA. The unincubated samples were treated with 2.0ml of 10% TCA prior to the addition of the enzyme source. The contents of both incubated and unincubated samples were filtered and the free amino acid content was determined in the filtrates. To 0.2 ml of the filtrate 2.0 ml of ninhydrin reagent was added and heated in a boiling water bath for 5 min and then cooled. The volume was made upto 10 ml with distilled water. The colour absorbance was measured at 570 nm against a reagent blank in a spectrophotometer. All samples are corrected for zero time controls. The proteolytic activity is expressed as M of tyrosine/mg. protein/ hr.

#### **Aspartate Amino Transferase (AAT) and Alanine Amino Transferase (ALAT) :**

In the colorimetric method, these enzymes otherwise called Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) catalyse, the transfer of -amino groups from specific amino acids to -ketoglutaric acid to yield glutamic acid and oxaloacetic acid or pyruvic acid. The formed ketoacids were then determined calorimetrically by the method of<sup>7</sup>.

#### **Estimation of Aspartate Amino Transferase (AAT)**

0.2 ml of the homogenate was pipetted into a clean test tube. To this, 100 M of L-aspartate, 100 M of phosphate buffer (pH7.5) and 2M of Ketoglutaric acid were added. The reaction was carried out at 37°C for 30 minutes. After incubation, 0.5ml of 2, 4-DNPH was added to arrest the reaction. After keeping the tubes for 20 minutes at room temperature, added 5 ml NaOH (1N) and mixed thoroughly. The colour developed was read in a spectrophotometer at 505 nm against the reagent blank. Zero time controls were maintained. The colour intensity was proportional to the transaminase activity and was expressed as M of pyruvate formed/mg. Protein/hr.

#### **Assay of alanine amino transaminase (ALAT)**

3% homogenates of control and experimental fish liver and muscle were prepared in phosphate buffer and centrifuged at 1500 rpm for 10 min. 0.2 ml of the supernatant was pipetted into a clean test tube. To this 100 M of L-alanine, 100 M of phosphate buffer (pH 7.5) and 2 M of - ketoglutaric acid were added. Then the reaction was carried out at 37°C for 30 minutes. After incubation, 0.5 ml of 2,4-dinitrophenyl hydrazine hydrochloride (DNPH) was added to arrest the reaction. After keeping the tubes for 20 minutes at room temperature, added 5 ml of NaOH (1N) and mixed thoroughly. The colour developed was read at 505 nm against a reagent blank in a spectrophotometer. Zero-time controls were also maintained. The colour intensity was proportional to the transaminase activity and was expressed as M of pyruvate formed/mg protein /hr.

#### **Estimation of glutamate dehydrogenase activity (GDH)**

The GDH activity was assayed by the method of<sup>8</sup>. 5% tissue homogenates were prepared in 0.25 M ice-cold sucrose solution. The homogenates were centrifuged at 1000 rpm for 15 min. The reaction mixtures in a final volume of contained 400 moles of sodium glutamate, 100 moles of sodium phosphate buffer (pH 7.4), 0.1moles nicotinamide adeninedinucleotide (NAD), 4 moles INT (2,4-iodophenol - and 3-4(4-nitrophenyl)- 5-phenyl tetrazolium chloride).

The reaction was initiated by the addition of 5.0 ml of the supernatant. The reaction mixture was incubated at 37°C for 30 minutes in a thermostatic water bath. Later, the reaction was stopped by the addition of 5.0 ml of glacial acetic acid. The formazan formed was extracted overnight at 50°C into 5 ml of toluene. The colour developed was measured at 495 nm in a spectrophotometer against toluene blank. The GDH activity was expressed as moles of formazan per mg protein per hour.

## RESULTS

The resulted data shows the changes in the muscle and liver protein metabolism of control and experimental fish batches. Control feed fed fish muscle of all species showed higher protein content compared to the liver. The muscle and liver of the control *H.molitrix* appeared to possess more proteins compared to other two species. The agrimin and fishmin fed fish species muscle and liver showed more percent elevations of their total protein content and the changes were found to be statistically significant over the control ( $P < 0.001$ ). Identical trends were also obtained for the muscle and livers free amino acid content. The changes in the control feed fed fishes, agrimin or fishmin fed fishes muscle and liver tissues protease activity were also presented. Protease activity was found to be more in the liver tissue. Agrimin or fishmin treatment enhanced the fish muscle and liver protease content and all the changes were found to be statistically significant over their corresponding control values. Liver tissue showed more AAT / ALAT/GDH levels. Agrimin and Fishmin fed fishes muscle and liver showed an increase in their AAT, ALAT and GDH activity levels. The muscle and liver tissues of the control feed fed fish batch appeared to possess higher AAT / ALAT/GDH levels compared to other species of fishes selected for the study.

## DISCUSSION

Many authors have demonstrated that increase in the body weight of animals was accompanied by the accumulation of various biochemical constituents like protein, FAA and enzymes like AAT and ALAT<sup>9,10,11,12</sup>. Proteins by far are most important group of macro molecular chemical substances which occupy a pivotal place in both structural and dynamic aspects of living systems. Further the catabolic products of proteins appear in the form of diet nitrogenous substances which are known to play a key role, in several key processes of animals. Metabolic response in proteins was considered to be one of the principle physiological events involved in the compensatory mechanism in terms of homeostasis under any stress condition<sup>13</sup>. Protein synthesis and degradation are reflected by changes in the protein composition<sup>14</sup>, protein of animal tissues are recognized to exist in a dynamic steady state undergoing continuous synthesis and degradation. Tissue proteins undergo a continuous process of renewal, referred to as 'turnover'. Protein concentrations are determined by the rates of degradation and synthesis both being regulatory in nature. Proteins must be continuously supplied to the organisms for growth and are to be maintained at constant levels<sup>15, 16</sup>. Further changes in protein concentrations probably reflect numerous physiological changes going on in the organism during growth period. Species - specific variations in the ontogenic pattern of various biochemical constituents are essential features of animal development. Various biochemical constituents like total and soluble proteins, FAA, AAT, ALAT, GDH Protease activities have been examined in tissues of various fish species under different conditions. The amino acids are the building blocks of proteins. The levels of amino acids show variations during different stages of fish development. Amino transferases catalyse the inter conversion of amino acids to Keto acids and vice versa. Amino transferase activity was in number of fish species during different developmental stages of fishes (Rangacharyulu et al., 2002) The major transmission reactions are performed by alanine amino transferase (Lehringer, 1993). Proteases are the most commonly found digestive systems in fishes (Chakrabarti, 1998) several factors responsible for the secretion of the proteolytic enzymes have been investigated by various authors<sup>17,18,19</sup>. In view of the key role played by proteins in the general metabolism of fishes, the author tried to investigate certain aspects of protein metabolism in the muscle and liver tissues of the selected fish species in the present study. Since liver is the major metabolic seat of the animal and the muscle being the vital part of the fish, these two tissues are conveniently selected by the author for biochemical studies.

The total proteins and amino acid contents registered an increase in the muscle and liver tissues of agrimin and fishmin fed fish species in the present study. Proteins are the chief organic constituents of the body. The macromolecules are concerned with the regulation of all biochemical events in the organism. Elevation in the proteins of agrimin and fishmin fed fish species muscle and liver is an indication of high protein content in these tissues due to the feeds used in the present investigation. Further agrimin fed fish tissues appeared to exhibit more protein content compared to fishmin fed ones. Free amino acids are not only the building blocks of all proteins but also the important constituents of fish nutrition (Rangacharyulu *et al.*, 2002). The changes in the fine amino acids can be correlated with the changes in the protein synthesis. The increase in the titers of free amino acids and those in the proteins in tissues of agrimin and fishmin fed fish tissues reflect the prevalence of both protein and amino acid synthesis. Supplementary activity seems to be predominant over utilization. The results observed for proteins and amino acids of the agrimin or fishmin fed fish tissues also suggest that the fish tissues are metabolically more active than the control feed fed ones and evidenced by the presence of increased levels of proteins and total free amino acids under agrimin and fishmin stress. This metabolic predominance of protein synthesis over proteolysis has greater significance in the fish tissues, since this situation denotes that agrimin or fishmin fed fish tissues improve their tissue protein content enormously compared to the control ones. Besides their role in protein synthesis, amino acids can influence various metabolic functions during fish growth. They are known to act as precursors of carbohydrate metabolism by positively influencing the transamination of aspartate and alanine which provide oxaloacetate and pyruvate to citric acid cycle. FAA are also implicated in lipogenesis<sup>20</sup>, maintenance in lipogenesis<sup>21</sup>, energy metabolism<sup>22</sup>, production of Gamma amino butyric acid (GABA)<sup>23</sup> and in the formation of haemocytes in the blood<sup>24</sup>. The amino acids may aid in any one of these or more physiological activities. Based on the results obtained in proteins and fine amino acids in tissues of the fish species selected for the study it may be construed that agrimin and fishmin might be acting as enhancers for the above stated roles of proteins and amino acids. Proteases are the most commonly found enzymes in fishes. Several factors responsible for the secretion of the proteolytic enzymes have been investigated by<sup>25</sup>. As observed in the present investigation and increase in agrimin and fishmin intakes the muscle and liver protease activity in the experimental fishes reflect a state of breakdown of proteins resulting in the formation of total Free amino acids. This might be due to inconsonance with the metabolic needs. Amino transferases operate at the cross over points between carbohydrate and protein metabolism by interconverting strategic cross over metabolites like -ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and tumerate on the other. Transaminases convert amino acids into Keto acids to be utilized for energy production, it can be envisaged that agrimin is more effective than fishmin in stimulating the proteolytic behaviours of the three fish species muscle and liver tissues, where these tissues showed more AAT, ALAT and GDH activities indicating more breakdown of proteins into FAA which in turn be fed into TCA cycle and into other metabolic pathways. From the current study, it is also evident from the results that both transaminases are active in the control group of tissues. Since ALAT promotes the production of pyruvate, it can be taken as a source of energy yielding metabolic and AAT activity indicates the production of oxaloacetate of TCA cycle and based on the results it can safely be concluded that the above cited situation is pronounced more in agrimin fed liver and muscle tissues of the three fish species selected for the study. AAT at any time represent channeling of FAA directly into TCA cycle thus enhancing energy yield through aerobic metabolism. Since the FAA pool is high in agrimin and fishmin fed fish tissues, it is likely that FAA may contribute to a greater extent (through activity of ALAT) towards transamination to step up gluconeogenesis in the muscle and liver tissues of all three fish species fed on agrimin or fishmin. This however is further investigated and related data form the content of further chooters of this dissertation.

In the present study, the agrimin or fishmin fed fish species muscle and liver tissues showed elevated activity levels of their GDH activity. GDH is an enzyme of great importance in the intermediary metabolism of amino acids. It is generally acts on the glutamic acid.

The dicarboxylic acid or its amide glutamate and -Ketoglutarate as such a link between amino acid and carbohydrate metabolism wherein NAD is always is required and is responsible for the ammonia formation in the animal tissues. In other words, glutamate and GDH have a unique role in amino group transfer. It is through this enzyme that the -Ketoglutarate is made available for the citric acid cycle, at the same time from ATP to release ammonia. The enhanced GDH activity in the muscle and liver tissues of three fish species fed on agrimin or fishmin indicates increased oxidation of glutamate. The rate of -Ketoglutarate as a substrate for sperm mobility was demonstrated at least in insects<sup>26, 27</sup>. Higher the-Ketoglutarate and higher the mobility of spermatozoa lead to facilitate higher choice of fertilization rate and thus higher fecundity and viability. Thus, the results obtained in the present investigation showed that both the aerobic and anaerobic metabolisms were speeded up due to agrimin and fishmin feeding of the three fish species and further it can be stated that agrimin and fishmin have triggered the intake of hexoses to glycolytic and Krebs cycle. Thus agrimin and fishmin feeding has cooperative interaction with the biochemical mechanism of protein synthesis in the muscle and liver tissues including oxidative metabolism.

**Effect of Agrimin & Fishmin on Muscle and Liver tissue total proteins levels of *H.molitrix*, *C.carpio*, *C.idella***

(Value expressed as mg/gm wet wt. tissue) Name of the parameter

Name of the Feed	Name of the parameter					
	Total proteins					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	3.62	0.92	3.91	0.97	3.46	0.95
SD	□0.085	□0.22	□0.036	□0.052	□0.069	□0.24
PC						
T						
Control Feed + Agrimin						
AV	4.96	1.04	4.36	1.13	3.81	0.99
SD	□0.17	□0.23	□0.069	□0.56	□0.423	□0.19
PC	9.39	14.34	11.50	16.49	10.11	4.21
T	*	*	*	*	*	*
Control feed + fishmin						
AV	4.82	1.07	4.02	1.05	3.52	0.96
SD	□0.66	□0.52	□0.34	□0.082	□0.34	□0.037
PC	5.52	5.43	2.81	8.24	1.73	0
T	*	*	*	*	*	N.S.

Each value is the mean □SD of 7 samples

AV – Average,

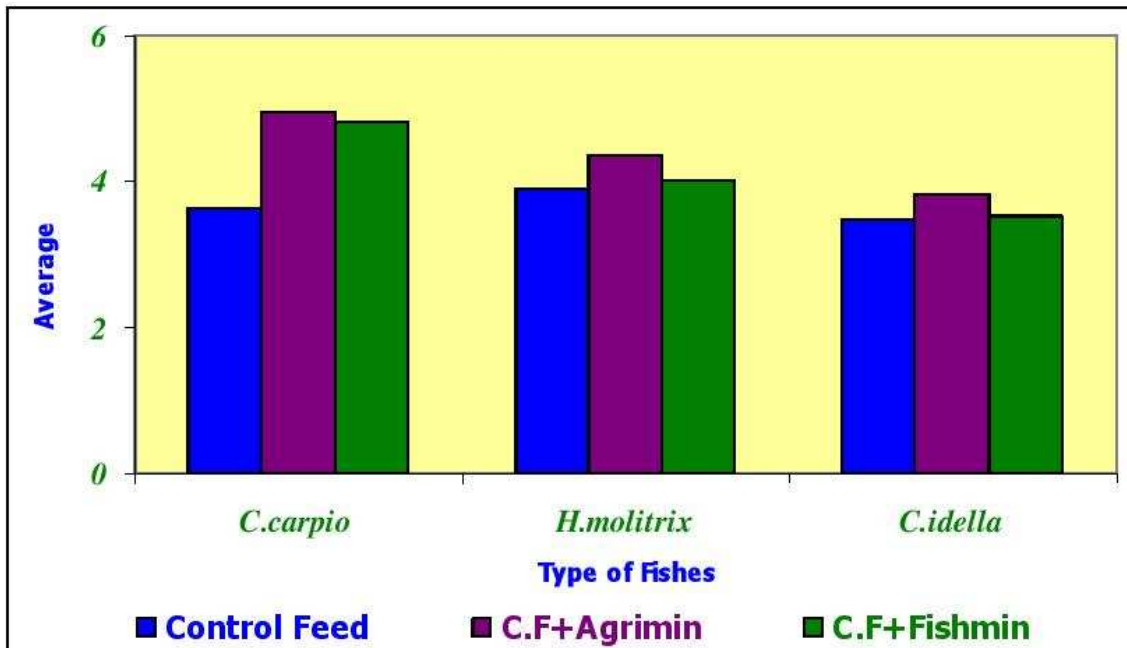
SD – Standard Deviation,

PC – Percentage change over the control;

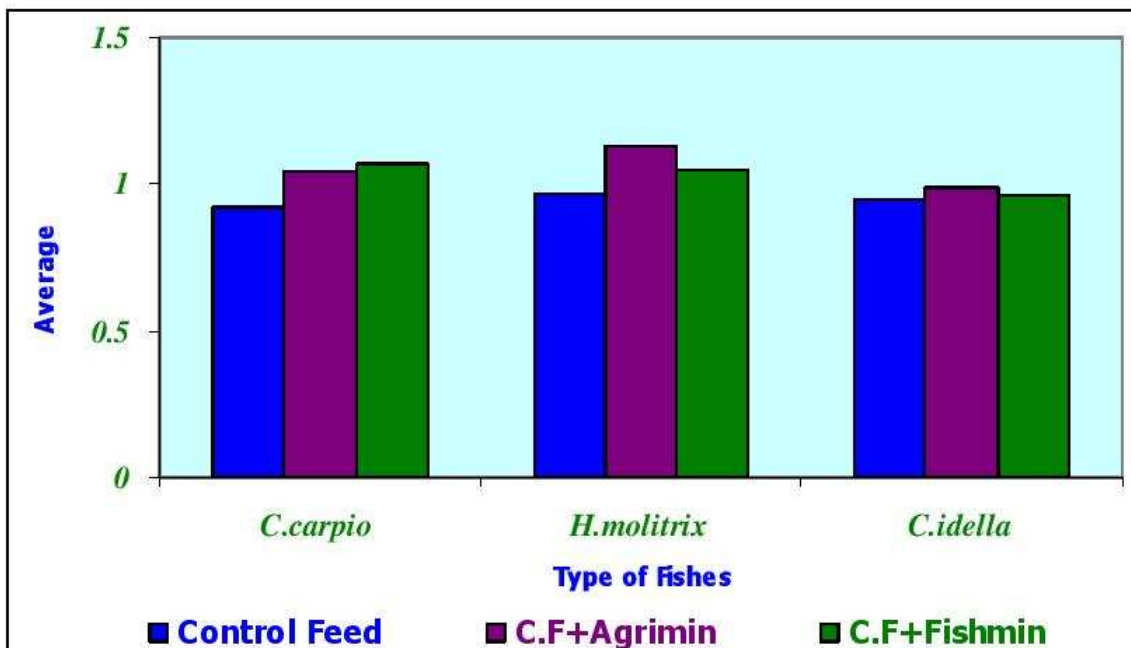
\* – P<0.001,

N.S. – Not significant

**Impact of Agrimin and Fishmin on the Total Proteins in Muscle of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



**Impact of Agrimin and Fishmin on the Total Proteins in Liver of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



**Effect of Agrimin & Fishmin on Muscle and Liver tissue free amino acids (FAA) levels of  
*H. molitrix*, *C. carpio*, *C. idella*  
(Value expressed ad mg/gm wet wt. tissue)**

Name of the Feed	Name of the parameter					
	Free amino acids					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV						
SD	294.86	73.85	301.58	75.45	300.55	72.62
PC	□3.74	□2.16	□4.79	□1.22	□1.69	□0.96
T						
Control Feed + Agrimin						
AV	324.29	81.05	331.84	83.23	330.84	79.95
SD	□1.26	□2.15	□5.69	□2.14	□7.16	□2.14
PC	9.98	9.74	10.03	10.31	10.17	10.04
T	*	*	*	*	*	*
Control feed + fishmin						
AV	309.53	77.42	316.69	79.24	316.46	77.52
SD	□6.19	□2.15	□1.67	□0.79	□4.14	□0.069
PC	4.79	4.83	5.01	5.09	5.27	6.74
T	*	*	*	*	*	*

Each value is the mean □SD of 7 samples

AV – Average,

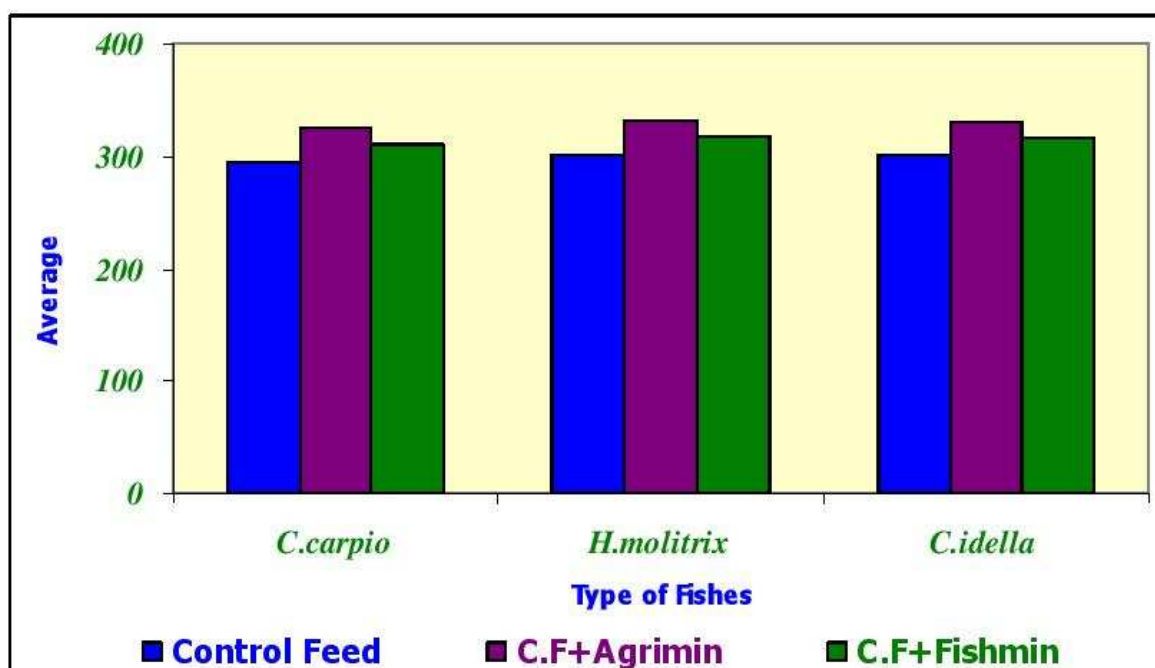
SD – Standard Deviation,

PC – Percentage change over the control ;

\* – P<0.001,

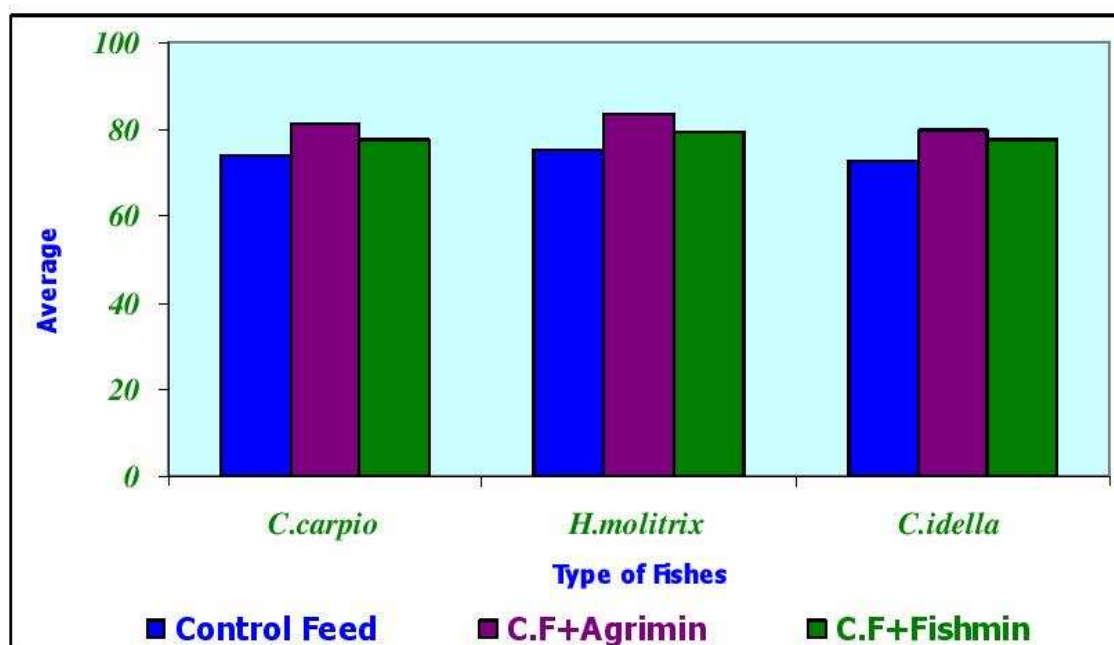
N.S. – Not significant

**Impact of Agrimin and Fishmin on the Free amino acids in Muscle of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**





**Impact of Agrimin and Fishmin on the Free amino acids in Liver of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



**Effect of Agrimin & Fishmin on Muscle and Liver tissue protease levels of *H.molitrix*, *C.carpio*, *C.idella* (Value expressed as moles of tyrosine equivalent /mg protein/hour)**

Name of the Feed	Name of the parameter					
	Protease					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.493	0.911	0.512	1.727	0.372	0.764
SD	□0.006	□0.007	□0.021	□0.042	□0.009	□0.24
PC						
T						
Control Feed + Agrimin						
AV	0.626	1.236	0.732	1.99	0.41	0.923
SD	□0.022	□0.045	□0.067	□0.034	□0.019	□0.36
PC	26.97	35.67	42.96	15.22	10.21	20.81
T	*	*	*	*	*	*
Control feed + fishmin						
AV	0.603	1.215	0.724	1.451	0.459	0.952
SD	□0.082	□0.14	□0.026	□0.079	□0.022	□0.059
PC	22.31	33.36	21.2	27.6	23.38	18.8
T	*	*	*	*	*	*

Each value is the mean □SD of 7 samples

AV – Average,

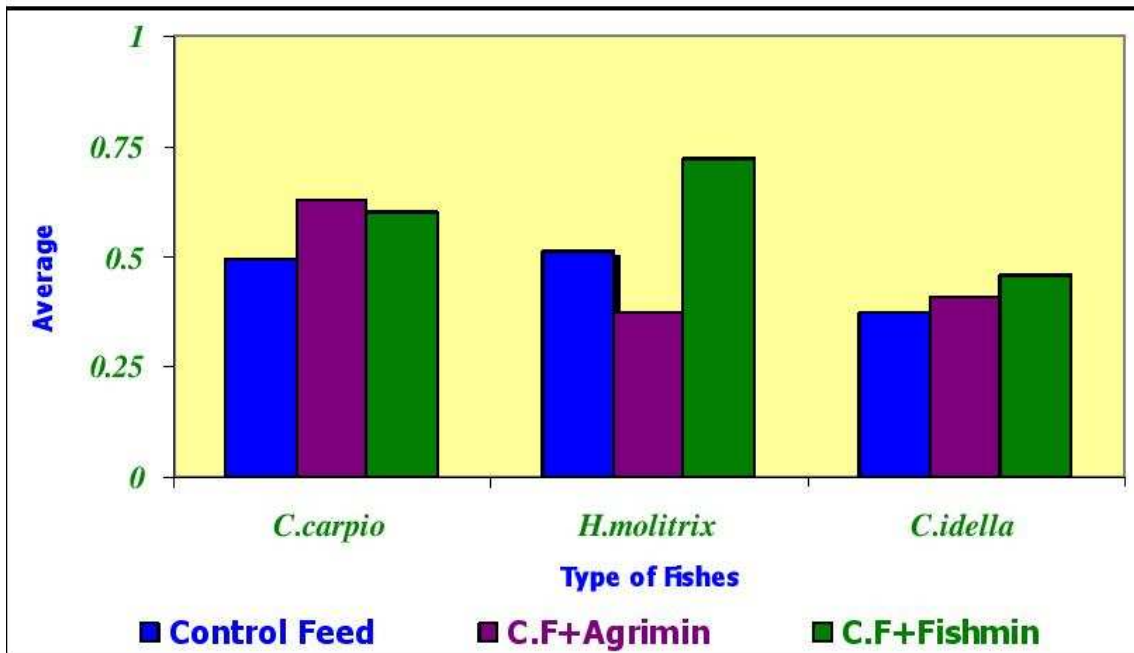
SD – Standard Deviation,

PC – Percentage change over the control ;

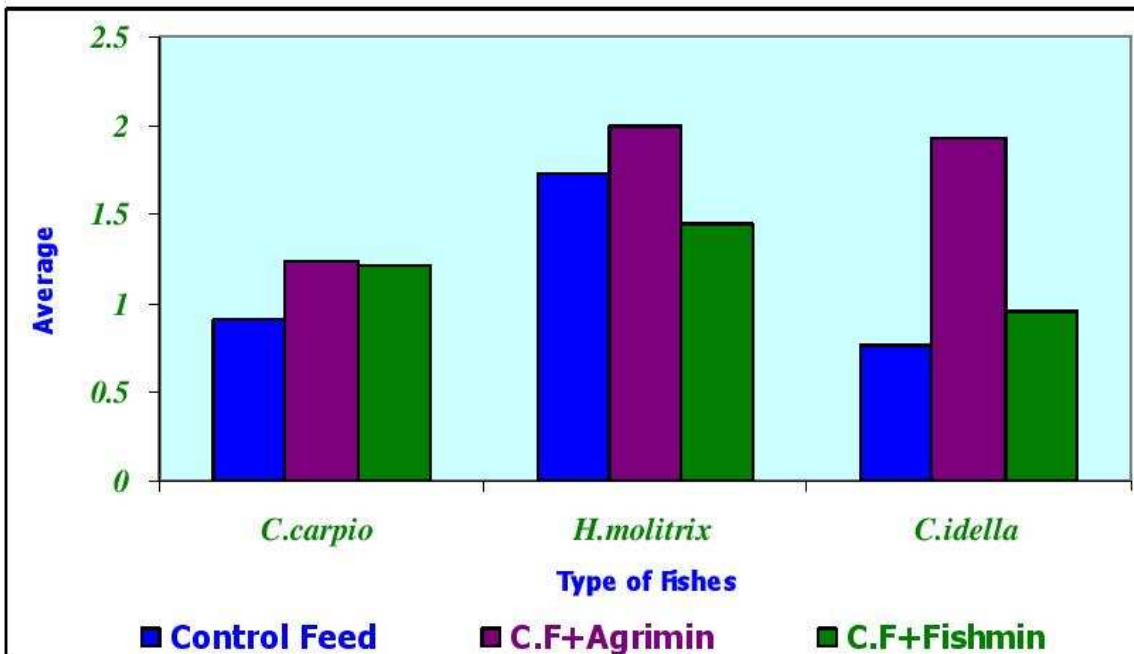
\* – P<0.001,

N.S. – Not significant

Impact of Agrimin and Fishmin on the *Protease in Muscle* of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.



Impact of Agrimin and Fishmin on the *Protease in Liver* of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.



**Effect of Agrimin & Fishmin on Muscle and Liver tissue Aspartate amino transferase (AAT) levels of  
*H. molitrix*, *C. carpio*, *C. idella***

(Value expressed as moles of Pyruvate formed /mg protein/hour)

Name of the Feed	Name of the parameter					
	Aspartate Amino Transferase (AAT)					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV						
SD	0.541	0.635	0.636	0.753	0.420	0.539
PC	□0.62	□0.41	□0.036	□0.33	□0.21	□0.034
T						
Control Feed + Agrimin						
AV	0.722	0.852	0.950	0.973	0.494	0.752
SD	□1.22	□0.037	□0.025	□0.049	□0.036	□0.21
PC	33.45	34.17	49.37	28.87	17.6	21.30
T	*	*	*	*	*	*
Control feed + fishmin						
AV	0.693	0.72	0.824	0.939	0.512	0.620
SD	□0.077	□0.045	□0.21	□0.064	□0.016	□0.022
PC	28.09	14.48	29.55	24.37	21.90	15.02
T	*	*	*	*	*	*

Each value is the mean □SD of 7 samples

AV – Average,

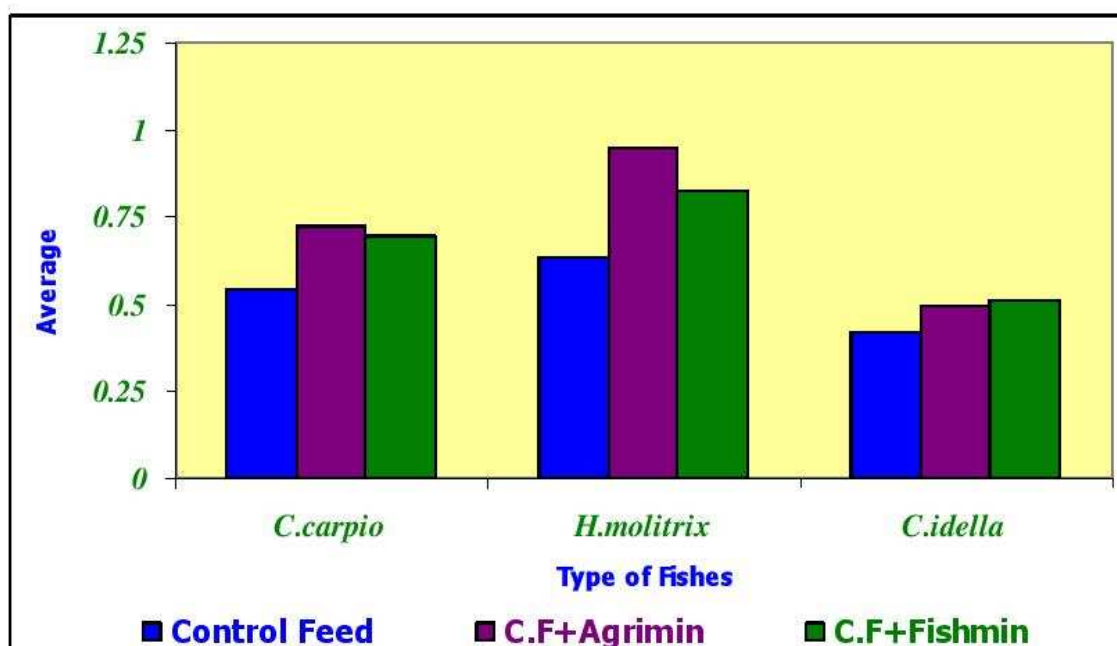
SD – Standard Deviation,

PC – Percentage change over the control

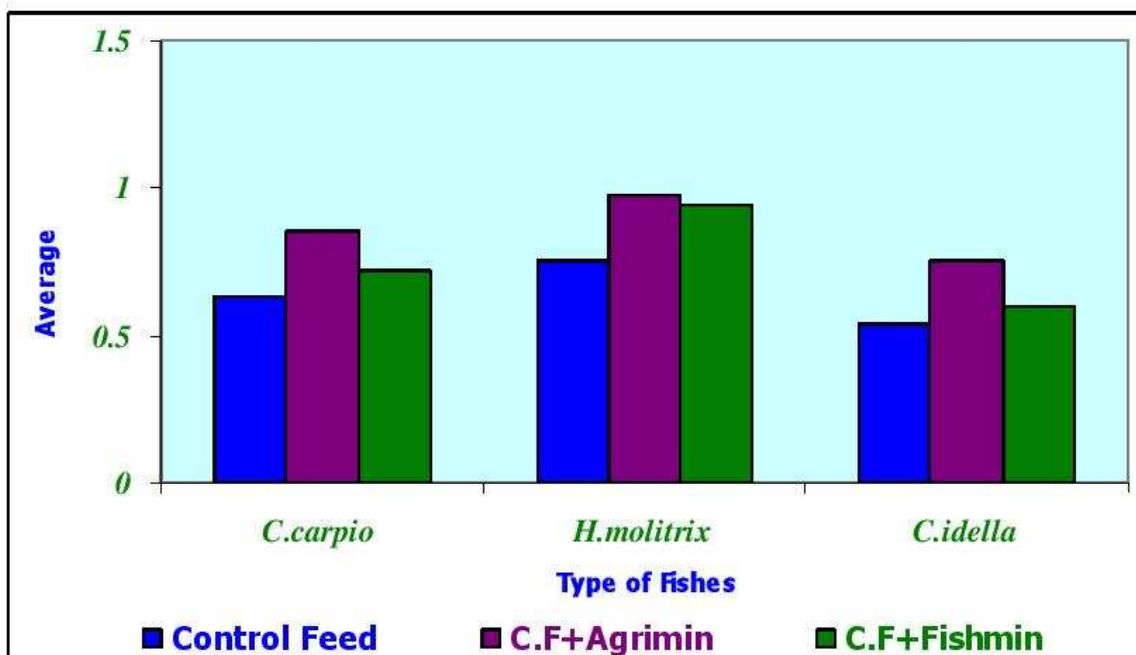
\* – P<0.001,

N.S. – Not significant

**Impact of Agrimin and Fishmin on the Aspartate amino transferase (AAT) in Muscle of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



**Impact of Agrimin and Fishmin on the Asparate amino transferase (AAT) in Liver of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



**Effect of Agrimin & Fishmin on Muscle and Liver tissue Alanine Amino Transferase (ALAT) levels of *H. molitrix*, *C. carpio*, *C. idella* (Value expressed as moles of Pyruvate formed /mg protein/hour)**

Name of the Feed	Name of the parameter					
	Alanine Amino Transferase (ALAT)					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.605	1.211	0.852	1.454	0.552	0.926
SD	□0.024	□0.067	□0.050	□0.067	□0.033	□0.0927
PC						
T						
Control Feed + Agrimin						
AV	0.856	1.367	0.992	1.824	0.713	0.984
SD	□0.052	□0.032	□0.041	□0.74	□0.082	□0.041
PC	41.48	12.88	16.43	25.44	29.16	6.26
T	*	*	*	*	*	*
Control feed + fishmin						
AV	0.784	1.224	0.968	1.675	0.746	1.051
SD	□0.074	□0.34	□0.044	□0.079	□0.16	□0.24
PC	17.90	1.07	13.61	15.19	35.14	13.49
T	*	*	*	*	*	*

Each value is the mean □SD of 7 samples

AV – Average,

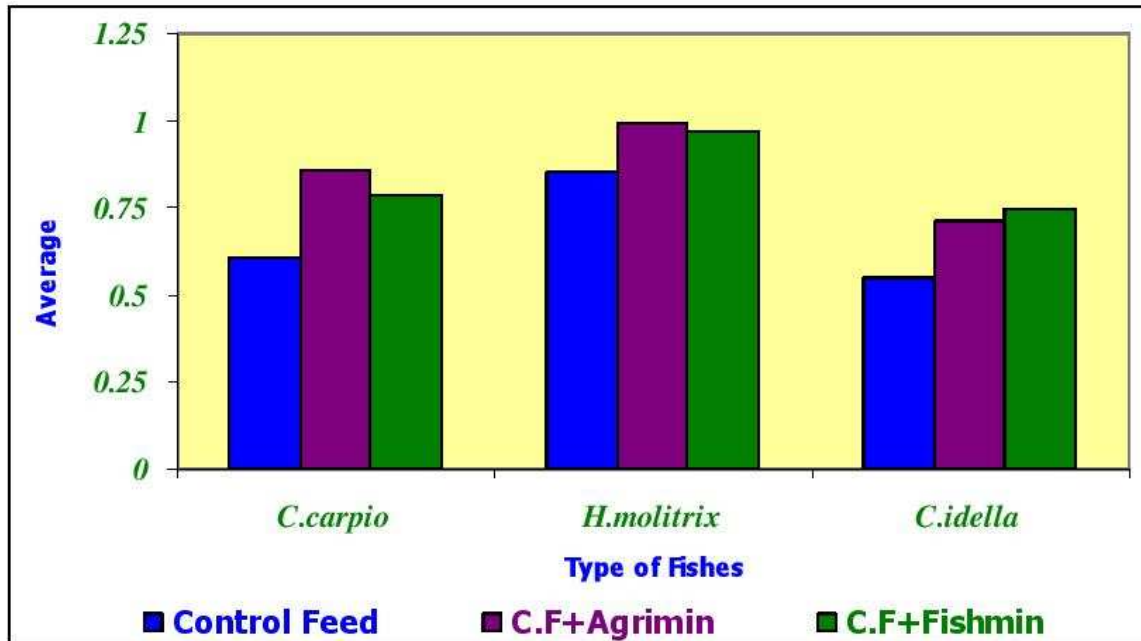
SD – Standard Deviation,

PC – Percentage change over the control;

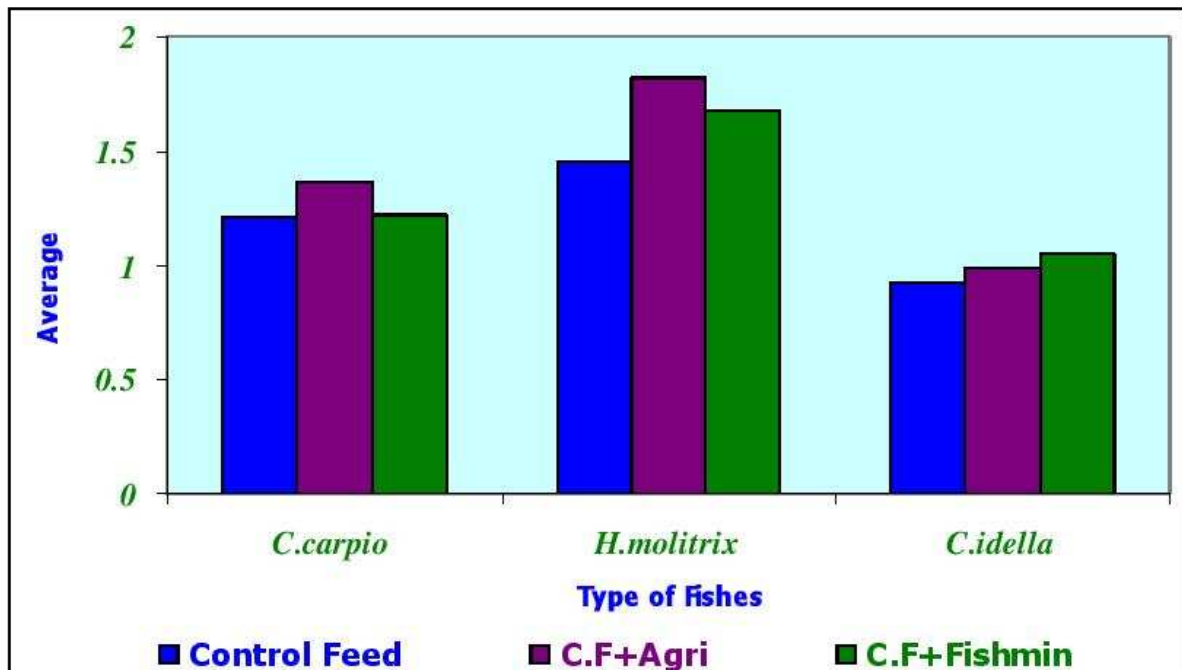
\* – P<0.001,

N.S. – Not significant

Impact of Agrimin and Fishmin on the *Alanine amino transferase (ALAT)* in Muscle of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.



Impact of Agrimin and Fishmin on the *Alanine amino transferase (ALAT)* in Liver of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.



**Effect of Agrimin & Fishmin on Muscle and Liver tissue (GDH) levels of *H.molitrix*, *C.carpio*, *C.idella* (Value expressed as mg/gm wet wt. Tissue)**

Name of the Feed	Name of the parameter					
	GDH					
	<i>Cyprinus carpio</i>		<i>Hypophthalmichthys molitrix</i>		<i>Ctenopharyngodon idella</i>	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.383	0.838	0.423	1.226	0.369	0.748
SD	□0.037	□0.046	□0.11	□0.021	□0.022	□0.006
PC						
T						
Control Feed + Agrimin						
AV	0.912	1.820	0.691	1.627	0.824	0.886
SD	□0.052	□0.062	□0.036	□0.31	□0.075	□0.025
PC	138.12	117.18	63.35	32.70	123.30	18.44
T	*	*	*	*	*	*
Control feed + fishmin						
AV	0.772	1.055	0.620	1.52	0.613	0.846
SD	□0.041	□0.034	□0.044	□0.11	□0.027	□0.034
PC	101.56	25.89	46.57	23.98	66.12	13.10
T	*	*	*	*	*	*

Each value is the mean □SD of 7 samples

AV – Average,

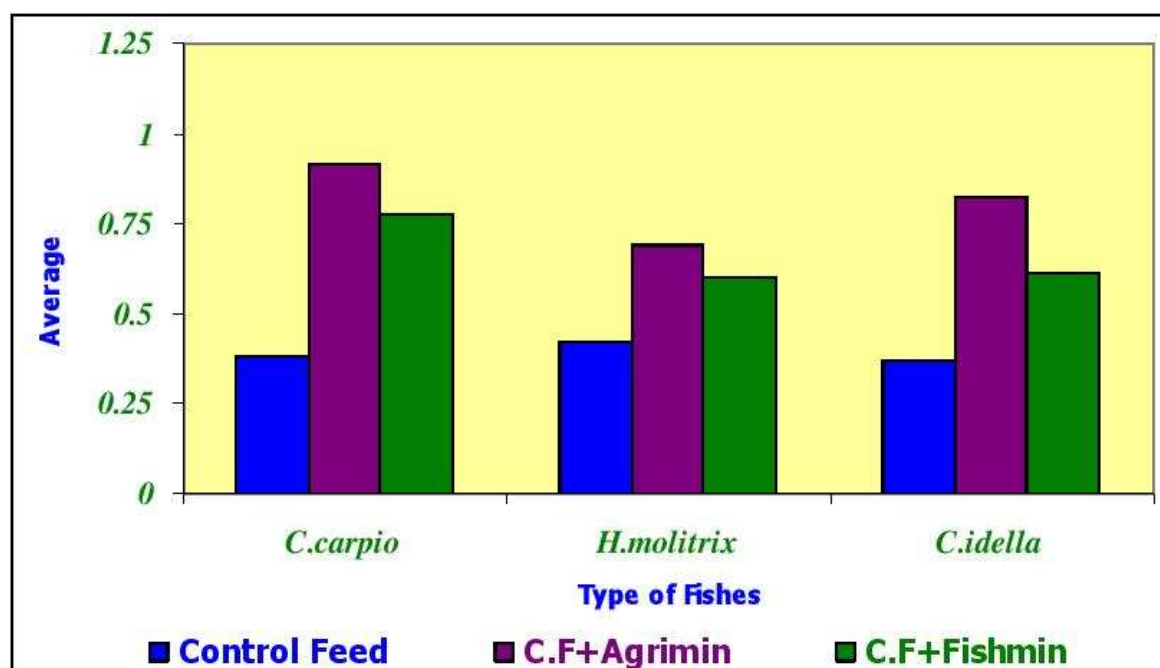
SD – Standard Deviation,

PC – Percentage change over the control ;

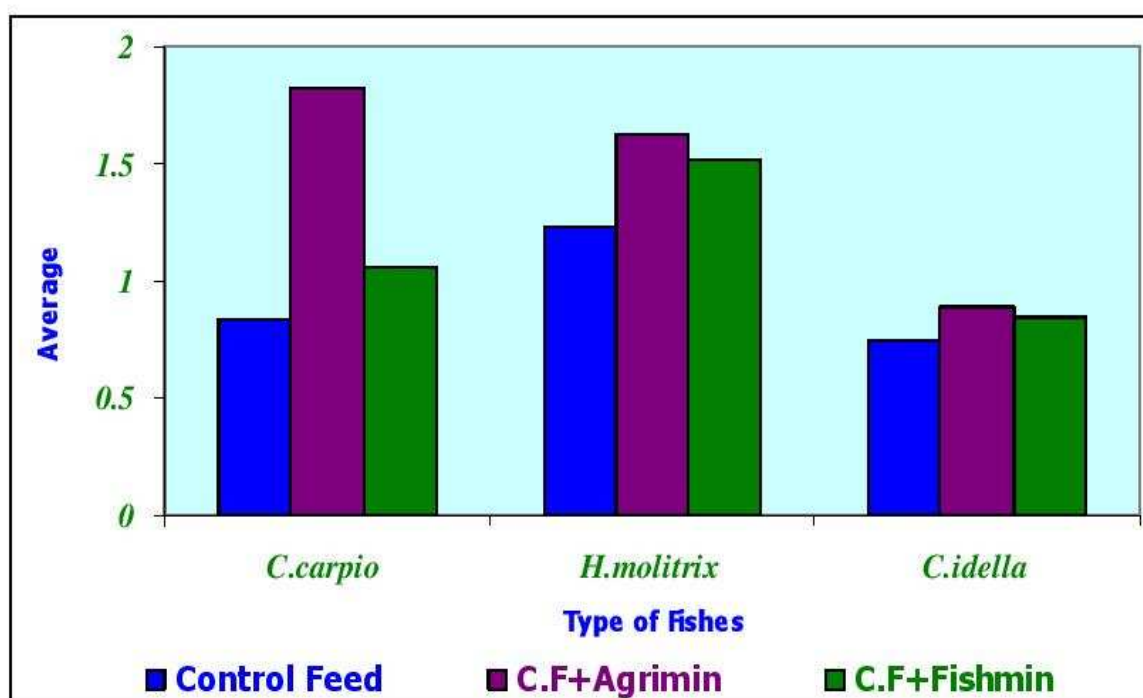
\* – P<0.001,

N.S. – Not significant

**Impact of Agrimin and Fishmin on the *Glutamate Dehydrogenase (GDH)* in Muscle of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.**



## Impact of Agrimin and Fishmin on the *Glutamate Dehydrogenase (GDH)* in Liver of Selected Fish species *H.molitrix*, *C.carpio*, *C.idella*.



### CONCLUSION

The agrimin and fishmin fed fish species muscle and liver showed more percent elevations of their total protein content and the changes were found to be statistically significant over the control. Identical trends were also obtained for the muscle and livers free amino acid content. Agrimin or fishmin treatment enhanced the fish muscle and liver protease content and all the changes were found to be statistically significant over their corresponding control values. Liver tissue showed more AAT / ALAT/GDH levels. Agrimin and Fishmin fed fishes muscle and liver showed an increase in their AAT, ALAT and GDH activity levels. The muscle and liver tissues of the control feed fed fish batch appeared to possess higher AAT / ALAT/GDH levels compared to other species of fishes selected for the study.

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