Biolife 1(3):-99-105
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE ORIGINAL ARTICLE

IMPACT OF AGRIMIN AND FISHMIN ON THE ASPECTS OF PROTEIN METABOLIC FROFILES IN DIFFERENT FISH (*C.CATLA, L. ROHITA, C.MRIGALA)* SPECIES

[1]Chennaiah K, [1]Sivasankar R and [2]Sugunakar Y.J

[1]Department of Zoology, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.
[2]Department of Biotechnology, JNTU, Pulivendula.

E-mail: chennaiahk@gmail.com

ABSTRACT

The present study is aimed at investigating the effect of selective Synthetic feed like Agrimin and Fishmin on protein metabolic profiles of the cultivable fish species like Catla catla, Labeo rohita, Cirrhinus mrigala. The fishes selected for the study shall be divided into two groups viz. control group and experimental group:age,two years .The control group of fishes shall be fed with control feed i.e. Groundnut cake, rice bran. The experimental group of fishes shall further be divided into two groups, Agrimin and Fishmin which are commercially available, have been selected for the study. The first group of experimental fish shall be fed with control feed mixed with Agrimin. The second group of experimental fish shall be fed with control feed mixed with fishmin. The two groups of experimental fish shall be fed twice a day at 10 a.m. and at 5 p.m. The exposure period selected for the study is 30 days,after 30 days the fishes were killed and isolated the tissues like muscle and liver at 4^0C and estimated the total proteins, free amino acids and proteases. Protease activity was found to be more in the liver tissue. The agrimin and fishmin fed fish species muscle and liver showed more percent elevations of their total protein 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.

Key words : Agrimin, Fishmin, Protein profiles, Fish species

.

INTRODUCTION

Proteins are the most characteristic chemical compounds found in the living cell. Proteins include several important cell constituents such as enzymes, peptide hormones, antibodies, transport molecules and components of cell skeleton including cell wall. 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 $1/5^{th}$of the animal body on the fresh weight basis (Swaminathan, 1983). Protein budge of the cell can be taken as an important diagnostic tool in evaluating its physical standards (Young, 1970). 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 (Rao and Vijayaraghavan, 1984; Zeitler, *et al.*, 1984). 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 of 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 (Seshalatha, 2003).

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, osoleucine, 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. (Love, 1980).

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 (Singh and Rastogi, 2002). 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 Indian Major carp *Labeo rohita, C.mrigala, C.catla.*

MATERIAL AND METHODS

Plane of work:

The fishes selected for the study shall be divided into two groups viz. control group and experimental group:age,two years .The control group of fishes shall be fed with control feed i.e. Groundnut cake, rice bran. The experimental group of fishes shall further be divided into two groups, Agrimin and Fishmin which are commercially available, have been selected for the study. The first group of experimental fish shall be fed with control feed mixed with Agrimin. The second group of experimental fish shall be fed with control feed mixed with fishmin. The two groups of experimental fish shall be fed twice a day at 10 a.m. and at 5 p.m. The exposure period selected for the study is 30 days,after 30 days the fishes were killed and isolated the tissues like muscle and liver at 4^0C .

Chemicals and synthetic 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).

1) Agrimin :

Agrimin is a product from Glaxo, Mumbai, India. A product with high quality supplement of minerals with essential amino acids for cattle

and fish feeding. Regular supplement of Agrimin helps in maintaining healthy growth and higher productivity.

Direction for use :
Can be mixed in Cattle and fish feed at the rate of 1-2% of feed (or) Large animals - 20 to 30 gms daily Small animals - 5 to 10 gms daily.

2. Fishmin :
Fishmin is a product from Arias Agro-vet industries Pvt. Ltd., Mumbai, India. A product with high quality supplement of minerals, mainly for aquatic animals. However, the author mixed fishmin with control feed at the rate of 1-2% for his study.

***Biochemical Investigation*:**
The total proteins in the tissues were estimated using the folin phenol reagent method as described by Lowry *et al*., (1951). The protein content is expressed as mg/g wet wt of the organ.Free amino acid levels in the tissues were estimated by the ninhydrin method as described by Moore and Stein, (1954). The free amino acid levels were expressed as mg amino acid nitrogen, released/gm. Wet wt of the tissue.Protease activity was estimated by the method of Moore and Stein, (1954). The proteolytic activity is expressed as M of tyrosine/mg. protein/ hr.

Statistical Analysis:
Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-value.

RESULTS

The data in the Table (1-3) 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 *C.catla* appeared to possess more proteins compared to other two species. (Table -1). 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 (Table 2).

The changes in the control feed fed fishes, agrimin or fishmin fed fishes muscle and liver tissues protease activity were presented in table 3. 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.

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 (Harihara Raju, 2001; Mamatha *et al*., 2002).

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 (Murray *et al*., 2000). Further the catabolic products of proteins appear in the form of di deet nitrogenous substances which are known to play a key role, in several key processes of animals (Nelson and Cox, 2000). 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 (Assem and Hanke, 1983). Protein synthesis and degradation are reflected by changes in the protein composition (Robert and Bocyuen, 1974), protein of animal tissues are recognised to exist in a dynamic steady state undergoing continuous synthesis and degradation (Goldberg, 1974).

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 (Segal *et al*., 1976). Proteins must be continuously supplied to the organisms for

Table 1: Effect of Agrimin & Fishmin on Muscle and Liver tissue total proteins levels of various fish species (Value expressed as mg/gm wet wt. tissue)

Name of the Feed	Name of the parameter					
	TOTAL PROTEINS					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	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	±0.5	±0.34	±0.08	±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

growth and are to be maintained at constant levels (Dunlop *et al*., 1978; Hershko and Ciechanover, 1982). Further changes in protein concentrations probably reflect numerous physiological changes going on in the organism during growth period (Annebond *et al*., 1993).

Species-specific variations in the ontogenic pattern of various biochemical constituents are essential features of animal development. Various biochemical constituents like total proteins, FAA and Protease activities have been examined in tissues of various fish species under different conditions.(Table 1-3).

The amino acids are the building blocks of proteins. The levels of amino acids show variations during different stages of fish development. Proteases are the most commonly found digestive systems in fishes (Chakrabarthi, 1998) several factors responsible for the secretion of the proteolytic enzymes have been investigated by various authors (Briegel and Lee, 1975; Sarangi *et al*., 1985). 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 (Table 1) and amino acid contents (Table 2) registered an increase in the muscle and liver tissues of agrimin and fishmin fed fish species in the present study. 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.

Table-2: Effect of Agrimin & Fishmin on Muscle and Liver tissue free amino acids (FAA) levels of various fish species. (Value expressed as mg/gm wet wt. tissue)

Name of the Feed	Name of the parameter					
	FREE AMINO ACIDS (FAA)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	294.86	73.85	301.58	75.45	300.55	72.62
SD	±3.74	±2.16	±4.79	±1.22	±1.69	±0.96
PC						
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.

Table-2: Effect of Agrimin & Fishmin on Muscle and Liver tissue free amino acids (FAA) levels of various fish species. (Value expressed as moles of tyrosine equivalent /mg protein/hour)

Name of the Feed	Name of the parameter					
	PROTEASE					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	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.

Free amino acids are not only the building blocks of all proteins but also the important constituents of fish nutritions (Rangacharyulu *et al*., 2000). 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. Synthetic 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.Proteases are the most commonly found enzymes in fishes (Rangacharyulu *et al*., 2002). Several factors responsible for the secretion of the proteolytic enzymes have been investigated by Briegel and Lea (1975).

As observed in the present investigation an increase in agrimin and fishmin intakes the muscle and liver protease activity in the experimental fishes (Table 3) reflect a state of breakdown of proteins resulting in the formation of total Free amino acids. This is shown in(Table 2). This might be due to inconsonance with the metabolic needs.

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 co-operative interaction with the biochemical mechanism of protein synthesis in the muscle and liver tissues including oxidative metabolism.

CONCLUSION

As observed in the present investigation an 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 is shown in. This might be due to inconsonance with the metabolic needs. 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 co-operative interaction with the biochemical mechanism of protein synthesis in the muscle and liver tissues including oxidative metabolism.

REFERENCES:

1. **Annebond, J. C.R.M Gonzalez. and B.P. Bradley**. (1993).Age-dependent. Expression of Proteins in the Cladoceran *Daphnia magna* under normal and heat stress conditions.
2. **Assem, H. and W. Hanke.** (1983) The significance of the amino acids during osmotic adjustment in telecast fish. I. Changes in the euryhaline *Sarotherodon mossambicus. Comp. Biochem. Physiol*. 74A : 531 – 536.
3. **Briegel, H. and A.O. Lea.** (1975). Relationships between protein and proteolytic activity in the mid gut of mosquitoes. *J. Insect. Physiol*. 21:1597-1604.
4. **Chakrabarthi, N.M**. (1998). Colour of *Labeo rohita*. In: Biology, culture and production of Indian major carps. *Narendra Publ. House*, New Delhi. Pp-89,131.
5. **Dunlop, D.S. W.V. Elden, and A. Lajtha**. (1978). Protein degradation rates in regions of the CNS in vivo during development. *Biochem. J*., 170:637.
6. **Goldberg, A.L.** (1974). Intracellular protein degradation in mammalian and bacterial cells. *Ann. Rev. Biochem*., 43: 835.

7. **Harihara Raju, A** (2001). Effect of turmeric on selected biochemical aspects of the silkworm *Bombyx mori* (L)., M.Phil., Dissertation submitted to Sri Venkateswar University, Tirupathi, India.
8. **Hershko, A. and A. Ciechanover**. (1982). Mechanisms of intercellular breakdown. *Ann. Rev. Biochem*, 51:335.
9. **Love, R.M.** (1980). The chemical biology of fishes. Academic Press, London. 2:943.
10. **Lowry, O.H. N.J. Rosenbrough, N.J. Farr, and R.J Randall** (1951). Protein Measurement with the folin-phenol reagent. *J. Biol. Chem.* 193:265-275.
11. **Mamatha, D.M., A.H.H. Raju., K.G. Reddy., P.S. Poornima., R.L.P. Reddy,. and M.R Rao.** (2002). "A juvenile hormone fenoxycarb can be used to boost more productivity in Sericulture practice : A short report : NSERD-II. Environmental awareness, Education and Management for Sustainable rural development. Dept. of Env. Sci., S.V. University, Tirupati, A.P. India. ABS NO. Vol. 1: pp. 62.
12. **Moore, S. and W.H. Stein** (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J.Biol.Chem*.211:907-913.
13. **Murray, R.K., D.K. Granner, P.A.Mayes, and V.W. Rodwell**. (2000). Harper's Biochemistry. 25th edition, McGraw – Hill companies, USA.
14. **Nagaraju Mareedu and Sunitha Devi Gudamani (2013).** Response of Skeletal Muscle Protein and Nucleic Acid Levels to Thyroxine Injection in Fish. *Biolife*.1(1):1-4.
15. **Nelson, D.L. and M.M. Cox** (2000). Lehninger principles of Biochemistry, third edition. MaCmillan Publishers, U.K.
16. **Rangacharyulu, P.V. B.N. Paul. S. Nandi, S. Sarkar and P.K. Mukhopadhyay**. (2002). Effect of different protein and energy levels on growth, nitrogen metabolism and body composition of rohu (*Labeo rohita*). *J. Aqua*., 8: 17-24.
17. **Rangacharyulu, P.V. K.J Rao. P.K Mukhopadhyay. and B.B Jana.** (2000). Evaluation of formulated diets for growth and nutrient utilization in Macrobrachium rosenbergii. National Workshop on 'Aquaculture of freshwater prawn'. Nellore. Andhra Pradesh, 8-9, February 2000.
18. **Rao, J.V.R. and S. Vijaya Raghavan** (1984). Biochemical alteration in the tissue of freshwater carnivorous teleost, *Anabas scandens* during nutritional stress through starvation and high carbohydrate loading. Symposium on feeding and nutrition in fish. Fish. Soc. Brit. Isles. Aberdeen (Anst).
19. **Robert, R.M. and Bocyuen** (1974). Chemical modification of plasma membrane polypeptides of cultural mammalian cell as an aid of studying protein turnover. *Biochem*., 13: 4846-4851.
20. **Sarangi, S.K.** (1985). Studies on the silkgland of *Bombyx mori.* A comparative analysis during fifth instar development. *Proc. Indian Acad. Sci. (Anim. Sci)* 94: 413-419.
21. **Segal, H.L. G.A. Dunway, and J.R Winkler**. (1976). The role of lysosomes in protein turnover in proteolysis and physiological regulation, (Vol.2, Ed: D.W. Robbins and K. Brew, Academic Press London), 287-308.
22. **Seshalatha, E. and P. Neeraja** (2003). Certain metabolic changes in fingerlings of *Cyprinus carpio* on ambient ammonia stress. *J. Aqua. Biol*. Vol. 18(2): 135-338
23. **Singh, H.S. and R.Rastogi** (2002). Osmotic homeostatis and free amino acids in snake headed murrel, *Channa punctatus* (Bloch.). *J.Aqua.Biol*.Vol.17(2):63-65.
24. **Swaminathan, M**. (1983). Handbook of food and nutrition, 3rd Edition, 22.
25. **Young, U.R.** (1970). Mammalian protein metabolism, Academic press, New York, U.S.A.
26. **Zeitler, M.H., M. Kirchgessner, and F.J. Schwarz** (1984). Effects of different protein and energy supplies on carcass composition of carp, *Cyprinus carpio. Aquaculture*. 36:37-48.