Carib.J.Sci.Tech

IMPACT OF SYNTHETIC FEED ADDITIVES ON AMINO ACID METABOLISM IN THE DIFFERENT FISH (*C.catla, L. rohita, C.mrigala)* SPECIES

Authors & Affiliation:
Venganna. P[1] RatnaSekar.P[2] Jagadish Naik.M*

[1&2]Department of Zoology, Acharya Nagarjuna University, Nagarjuna NagarGuntur, AndhraPradesh-522510 India.

.

KEY WORDS:

Agrimin, fishmen, AAT, ALAT, GDH and Fish species.

Corresponding Author:

M.Jagadish Naik

ISSN 0799-3757

http://caribjscitech.com/

Abstract:

The present study is aimed at investigating the effect of selective Synthetic feed like Agrimin and Fishmin on amino acid metabolism of the cultivable fish species like *Catla catla. Labeo rohita. Cirrhinus mrigala*. The fishes selected for the study are divided into two groups viz. control group and experimental groups .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 has been fed with control feed mixed with Agrimin. The second group of experimental fish has been 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 was selected for the study after 30 days the fishes were sacrificed and isolated the tissues like muscle and liver at 4^0C and assayed the activity of Asperate amino transferase (AAT), Alanine Amino Transferase (ALAT) and Glutamate dehydrogenase (GDH). Agrimin and Fishmin feed fed fishes like muscle and liver showed an increase in their AAT, ALAT and GDH activity levels.

INTRODUCTION

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 1/5thof 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 – e.g. 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). The use of high lipid diets in farmed fish can increase energy stored in adipose tissue with the consequence of excess fat deposition, which is generally not desirable in aquaculture products. Moreover, as a result of global limits on the supply of fish oil (2), there is a drive to replace fish oils with plant derived oils in aquaculture diets Javidi et al., 2004; Degrace et al., (2004). 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.*

METHODOLOGY:

Plan of work:

For the present study stocking / Breeders pond. Breeding tubs. Hatching tub and Nursery cum Rearing ponds were used at the Government fish farm at Nandyal(Kurnool District).Andhra Pradesh .India. The breeders were fed with

shell, rice bran and ground nut oil cake regularly at the rate of 2% body weight of the fish. The fishes selected for the study shall be divided into two groups viz. control group and experimental groups .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 are 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 stored at - 80^0C and assay the activity of Asperate amino transferase(AAT), Alanine Amino Transferase (ALAT) and Glutamate dehydrogenase (GDH).

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. CDH (India).

1) Agrimin : Agrimin is a product from Glaxo company Mumbai. India. A product with high quality supplements 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. The author mixed fishmin with control feed at the rate of 1-2% for his study.

Biochemical Investigation:

Aspartate Amino Transferase (AAT) and Alanine Amino Transferase (ALAT) by the method of Reitman and Frankel (1957). The colour intensity was proportional to the transaminase activity and was expressed as M of pyruvate formed/mg. Protein/hr. The GDH activity was assayed by the method of Lee and Lardy (1965). The GDH activity was expressed as moles of formazan per mg protein per hour.

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:

Liver tissue showed more AAT / ALAT/GDH levels (Table 1, 2 and 3). Agrimin and Fishmin feed to fishes and isolated the 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.(Table-1,3).

DISCUSSION:

Many authors have demonstrated that increase in the body weight of animals was accompanied by the accumulation of various biochemical constituents like protein, free amino acids (FAA) and enzymes like AAT and ALAT (Dhinakar, 1988; Hari Hara Raju, 2001; Mamatha *et al.,* 2002).

Free amino acids are not only the building blocks of all proteins but also the important constituents of fish nutritions (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. 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.

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 aspirate and alamine which provide oxaloacetate and pyruvate to citric acid Cycle (Harper *et al.,* 1993). FAA is also implicated in lipogenesis (Pant and Jaiswal, 1981), energy metabolism (Parenty *et al.,* 1985), and production of Gamma amino butyric acid (GABA) (Henry *et al.,* 1985) and in the formation of haemocytes in the blood (Robinson *et al.,* 1981). 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 (Rangacharyulu *et al.,* 2002). Several factors responsible for the secretion of the proteolytic enzymes have been investigated by Briegel and Lea (1975).

Aminotransferases operate at the cross over points between carbohydrate and protein metabolism by interconverting strategic cross over metabolites like -ketoglutarate, pyruvate and oxateacetate on one hand and alanine, aspartate and tumerate on the other (Nelson and Cox, 2000; Nagaraju *et al*, 2012). Transaminases convert amino acids into Keto acids to be utilized for energy production. In view of this, and based on the experimental results presented in (Table1&2), it can be envisaged that agrimin is more effective than fishmin in stimulating the proteolytic behaviors 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.

Conclusion: As observed in the present investigation the synthetic feed selected gives the good nutritional status through examining the tissues in fishes .The amino acids 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. 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 enhancing.Agrimin and fishmin feeding has co-operative interaction with the biochemical mechanism of protein synthesis in the muscle and liver tissues including oxidative metabolism.

Table -1: Effect of Agrimin & Fishmin on Muscle and Liver tissue Asperate amino transferase (AAT) levels of various fish species. (Value expressed as moles of Pyruvate formed /mg protein/hour).

Name of the Feed	Name of the parameter					
	Asperate Amino Transferase (AAT)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	1. Muscle	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**
Control Feed AV SD PC t	0.541 ±0.62	0.635 ±0.41	0.636 ±0.036	0.753 ±0.33	0.420 ±0.21	0.539 ±0.034
Control Feed + Agrimin AV SD PC t	0.722 ±1.22 33.45 *	0.852 ±0.037 34.17 *	0.950 ±0.025 49.37 *	0.973 ±0.049 28.87 *	0.494 ±0.036 17.6 *	0.752 ±0.21 21.30 *
Control feed + fishmin AV SD PC t	0.693 ±0.077 28.09 *	0.72 ±0.045 14.48 *	0.824 ±0.21 29.55 *	0.939 ±0.064 24.37 *	0.512 ±0.016 21.90 *	0.620 ±0.022 15.02 *

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 Alanine Amino Transferase (ALAT) levels of various fish species. (Value expressed as moles of Pyruvate formed /mg protein/hour).

Name of the Feed	Name of the parameter					
	Alanine Amino Transferase (ALAT)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	2. Muscle	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**
Control Feed AV SD PC t	0.605 ±0.024	1.211 ±0.067	0.852 ±0.050	1.454 ±0.067	0.552 ±0.033	0.926 ±0.0927
Control Feed + Agrimin AV SD PC t	0.856 ±0.052 41.48 *	1.367 ±0.032 12.88 *	0.992 ±0.041 16.43 *	1.824 ±0.74 25.44 *	0.713 ±0.082 29.16 *	0.984 ±0.041 6.26 *
Control feed + fishmin AV SD PC t	0.784 ±0.074 17.90 *	1.224 ±0.34 1.07 *	0.968 ±0.044 13.61 *	1.675 ±0.079 15.19 *	0.746 ±0.16 35.14 *	1.051 ±0.24 13.49 *

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 3: Effect of Agrimin & Fishmin on Muscle and Liver tissue Glutamate dehydrogenase (GDH) levels of various fish species .(Value expressed as mg/gm wet wt. tissue.

Name of the Feed	Name of the parameter					
	Glutamata dehydrogenase (GDH)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	3. Muscle	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**
Control Feed AV SD PC t	0.383 ±0.037	0.838 ±0.046	0.423 ±0.11	1.226 ±0.021	0.369 ±0.022	0.748 ±0.006
Control Feed + Agrimin AV SD PC t	0.912 ±0.052 138.12 *	1.820 ±0.062 117.18 *	0.691 ±0.036 63.35 *	1.627 ±0.31 32.70 *	0.824 ±0.075 123.30 *	0.886 ±0.025 18.44 *
Control feed + fishmin AV SD PC t	0.772 ±0.041 101.56 *	1.055 ±0.034 25.89 *	0.620 ±0.044 46.57 *	1.52 ±0.11 23.98 *	0.613 ±0.027 66.12 *	0.846 ±0.034 13.10 *

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.

References

1. Briegel, H. and Lea, A.O., 1975, Relationships between protein and proteolytic activity in the mid gut of mosquitoes. *J. Insect. Physiol*. 21:1597-1604.
2. Dhinakar, G.M.,1988, Studies on the effect of favourable and unfavourable seasons on the growth, physiology and fecundity of silkworm, *Bombyx mori*, L; Ph.D; thesis, S.V. University, Tirupathi, India.
3. Degrace P, Demizieux L, Gresti J, Chardigny JM, Sebedio JL, Clouet P.,2004, Hepatic steatosis not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10,cis-12-isomer of linoleic acid. *J. Nutr*. 134:861–7.
4. 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.
5. Harper, H.A; Rodwell, V.W. and Mayers, P.A., 1993, Review of physiologicalchemistry. 21st Ed. Lange Medical publications. Los Altos, California.
6. Henry, G; Trapido, R. and Morse, D.E.,1985, Diamino acids facilite GABA Induction of larval morphosis in a gastropod mollusk (*Holiotis rufescene*). *J. Comp. Physiol*. 155B : 403-414.
7. Javadi M, Beynen AC, Hovenier R, Lankhorst AE, Lemmens AG,Terpstra AHM, Geelen MJH.,2004, Prolonged feeding of mice with conjugated linoleic acid increases hepatic fatty acid synthesis relative to oxidation. *J Nutr Biochem*. 15:680–7.
8. Lee,Y.L and Lardy,A.,1965, Influence of thyroid hormones on phosphate dehydrogenanse and other dehydrogenases in various organs of the rat. *J. Biol. Chem*. 240: 1427 – 1432.
9. Love, R.M., 1980, The chemical biology of fishes. Academic Press, London. 2:943.
10. Mamatha, D.M; A.H.H Raju ; K.G Reddy; P.S Poornima; R.L PReddy, and Rao, M.R.,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.
11. Nagaraju Mareedu and Sunitha Devi Gudamani**.,** 2012, Response of skeletal muscle protein and nucleic acid levels to thyroxine injection in fish.*j. Biolife*, 1(1), 1-4.
12. Nelson, D.L. and Cox, M.M.,2000, Lehninger principles of Biochemistry, third edition. MaCmillan Publishers, U.K.
13. Osanai, M; Aigaki, T. and Kosuga, H., 1987a, Energy metabolism in the spermatophore of the silkmoth *Bombyx mori*, associated with accumulation of alanine derived from arginine. *Insect Biochm*. 17 : 71-75.
14. Rangacharyulu, P.V; B.N Paul, S. Nandi, S. Sarkar and Mukhopadhyay, P.K., 2002,E ffect of different protein and energy levels on growth, nitrogen metabolism and body composition of rohu (*Labeo rohita*). J. Aqua; 8: 17-24.
15. Rao, J.V.R; and Vijaya Raghavan,S.,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).
16. Reitman, S; Frankel, S.,1957, Calorimetric method of the determination of serum oxaloacetic and glutamic pyruvic transaminases. *An. J. Clini; Pathology*, 28: 53-56.
17. Seshalatha, E. and Neeraja, P.,2003, Certain metabolic changes in fingerlings of *Cyprinus carpio* on ambient ammonia stress. *J. Aqua. Biol. Vol*. 18(2): 135-338
18. Singh, H.S and Rastogi, R .,2002, Osmotic homeostatis and free amino acids in snake headed murrel, *Channa punctatus* (Bloch.). *J.Aqua.Biol.Vol*.17(2):63-65.
19. Swaminathan, M., 1983, Handbook of food and nutrition, 3rd Edition, 22.
20. Young, U.R .,1970, Mammalian protein metabolism, Academic press, New York, U.S.A.
21. Zeitler, M.H; Kirchgessner, M and Schwarz, F.J (1984). Effects of different protein and energy supplies on carcass composition of carp, *Cyprinus carpio. Aquaculture*. 36:37-48.