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Nutritional efficacy of Richmin and vanimin feed additives on amino acid metabolism in fish (*C. catla, L. rohita, C. mrigala*) species under field conditions

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Abstract
The present study is aimed at investigating the effect of selective Synthetic feed like Richmin and Vaniminon 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. Richmin and Vanimin which are commercially available have been selected for the study. The first group of experimental fish has been fed with control feed mixed with Richmin. The second group of experimental fish has been fed with control feed mixed with Vanimin. 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).Richmin and Vanimin feed fed fishes like muscle and liver showed an increase in their AAT, ALAT and GDH activity levels.

Keywords: richmin, vanimin AAT, ALAT, GDH and fish species

1. 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) [27]. Protein budge of the cell can be taken as an important diagnostic tool in evaluating its physical standards (Young., 1970) [28]. Proteins may be hydrolyzed 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 jar et al., 1984) [22]. 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 and Neeraja, 2003) [25]. 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 Rm., 1980) [14].

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) [26]. 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, there is a drive to replace fish oils with plant derived oils in aquaculture diets (Javadi and Degrace, 2004; Jagtap and Kulkarni., 2013) [10, 12]. 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 feed formulation of selected feeds having much nutritional impact like Richmin and Vanimin in Indian Major Carp *Labeo rohita, C. mrigala, C. catla.*

2. 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 Guntur (Guntur 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. Richmin and Vanimin which are commercially available are been selected for the study. The first group of experimental fish shall be fed with control feed mixed with Richmin. The second group of experimental fish shall be fed with control feed mixed with vanimin. 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).

Additives of Synthetic Feed

Richmin and Vaniminwhich are commercially available have been selected for the study. All other chemicals used are of technical grade from PVS laboratories, Vijayawada, Andhra Pradesh (India).

1. Richmin

Richmin is a product from PVS laboratories, Vijayawada, Andhra Pradesh India. A product with high quality supplements of minerals with essential amino acids for fish feeding. Regular supplement of Richmin helps in maintaining healthy growth and higher productivity

Direction for use

Can be mixed with 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. Vanimin

Vanimin is a product from PVS laboratories, Vijayawada, Andhra Pradesh 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 [19]. 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 [8]. The GDH activity was expressed as moles of formazan per mg protein per hour.

Estimation of Aspartate Amino Transferees (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°Cfor 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 370C 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 8. 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).

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.

3. Results

Liver tissue showed more AAT / ALAT/GDH levels (Table 1,

2 and 3). Richmin and vanimin 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).

Table 1: Efficacy of Richmin & Vanimin 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*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.531	0.645	0.626	0.743	0.420	0.549
SD	±0.62	±0.41	±0.036	±0.33	±0.21	±0.034
Control Feed + Richmin						
AV	0.723	0.853	0.950	0.973	0.493	0.751
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 + Vanimin						
AV	0.694	0.72	0.823	0.938	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.

Table 2: Efficacy of Richmin & Vanimin 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*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.604	1.211	0.852	1.453	0.552	0.925
SD	±0.024	±0.067	±0.050	±0.067	±0.033	±0.0927
Control Feed + Richmin						
AV	0.855	1.366	0.992	1.823	0.713	0.985
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 + Vanimin						
AV	0.784	1.223	0.969	1.674	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.

Table 3: Efficacy of Richmin & Vanimin 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					
	Glutamate dehydrogenase (GDH)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	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
Control Feed + Richmin						
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 + Vanimin						
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.

4. 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 and Harihararaju *et al.*, 2004) [3, 5].

Free amino acids are not only the building blocks of all proteins but also the important constituents of fish nutrition (Rangacharyulu *et al.,* 2002) [21]. 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 Richmin and Vanimin 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 richmin or vanminn 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 some synthetic feeds. This metabolic predominance of protein synthesis over proteolysis has greater significance in the fish tissues, since this situation denotes that richmin and vanimin 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 (Hharper*et al.,* 1993) [7]. FAA is also implicated in lipogenesis (Pant and Jaiswal, 1981), energy metabolism (Parenty *et al.,* 1985) [20], and production of Gamma amino butyric acid (GABA) and in the formation of haemocytes in the blood (Robinson *et al.,* 1981) [24]. 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 (Henry *et al.,* 1985) [8]. Several factors responsible for the secretion of the proteolytic enzymes have been investigated by (Briegel and lea 1975; Harikrishnan *et al.,* 2009;Janakiraman and Altaff 2014) [1, 6, 11].

Aminotransferases operate at the cross over points between carbohydrate and protein metabolism by inter converting strategic cross over metabolites like ∞-ketoglutarate, pyruvate and oxateacetate on one hand and alanine, aspartate and tumerate on the other (Nagarajum *et al*., 2012) [16]. 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.

5. 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 Nutritional ingredients of feed with additives feeding of the three fish species and further it can be stated that Richmin and Vanimin enhancing the metabolic and productive of the carps. Richmin and Vanimin feeding has co-operative interaction with the biochemical mechanism of protein synthesis in the muscle and liver tissues including oxidative metabolism.

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