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Impact of Agrimin And Fishmin on the Carbohydrate Metabolic Enzymes in the Muscle and Liver Tissue of the Different Fish (*C.Catla. L. Rohita. C.Mrigala*) Species

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ABSTRACT

The present study is aimed at investigating the effect of selective Synthetic feed like Agrimin and Fishmin on carbohydrate metabolic enzymes 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 assayed the activity of Aldolose. MDH (Malate dehydrogenase). SDH (Succinate dehydrogenase) and LDH (Lactate dehydrogenase). Agrimin and fishmin enhanced the various fish species muscle and liver tissues enzymes such as Aldolose, MDH, SDH and LDH activity levels.

Key words : Fish feed, Aldolose, MDH, SDH, LDH, and Fish species.

INTRODUCTION

The most important and characteristic element of living organisms is carbon. Carbon atoms participate in the formation of an almost infinite variety of molecules because of the ability of carbon atoms to combine with one another to form long chains and double covalent bonds. Carbohydrates are one such group of carbon compounds which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact. Exploit their rich supply of potential energy to maintain the life.

Carbohydrates constitute by far the greatest proportion of organic material on the face of the earth and the most abundant carbohydrate is cellulose which forms the main supporting structure of plants. They constitute important materials for the necessities of life such as food and clothing, housing and health. As reserve food material for plants. Starches are stored in grains. Tubers and roots and form the staple food and main energy sources of the poorer populations of the world. The sugars are found in the nectar of flowers and in fruits and milk.

Impairment of carbohydrate metabolism has been observed in a variety of physiological disorders and pathological conditions (Harper *et*

al., 1979). This may prove to be of negative survival value for the affected organisms. Investigations were conducted earlier on carbohydrate metabolism during pathological conditions in different animals following exposure of some kinds of pesticides (Dikshit *et al*., 1975). Glucose in the blood which shows most striking alternations in its concentration in response to change in environmental factors (Umminger 1975). More over in several fishes blood glucose level has been correlate to their level of activity and thus ultimately the level of blood glucose is attributed to and indirect level of B.M.R. in fishes (Umminger 1977).

Glucose is the principal sugar in blood of fishes. serving the tissues as a major metabolic fuel. Besides yielding energy through glycolysis and TCA cycle. pentose sugars are also formed in the hexose monophosphate shunt from glucose. which are important constituents of nucleotides. nucleic acids and many coenzymes. In general glucose level in the blood of circulating fluid is maintained in an animal through active absorption of glucose from the digested food stuffs. and also is formed from glycogen. amino acids and glycerol through glycogenolysis and gluconeogenesis under certain stress conditions. In several fishes blood glucose level has been correlated to their level of activity and hence to their level of metabolism. There are evidences that in fish's blood glucose level shows most striking alterations in response to the change in environmental factors (Umminger 1975; Hattinght 1977). The levels of it may even be affected under toxic stress. which reflects the variations in the entire carbohydrate metabolism. (Tewari *et al*., 1987). Blood glucose level has been reported as a reliable and sensitive indicator of environmental stress in fishes (Silbergeld 1974 & Nagaraju et al, 2012).

Glycogen commonly called as animal starch. is the main storage polysaccharide and a great source for blood glucose. Maintenance of glycogen reserves is one of the important features of the normal metabolism (Mong and Poland 1981). Alterations in liver and muscle glycogen under situations of stress have been reported. and a significant depletion if tissue glycogen is said to reflect the state of strenuous activity on the part of the fish (Tewari *et al*., 1987). In many of the fishes red muscle is known to be predominantly oxidative where as white muscle is known to be predominantly glycolytic (Gordon 1968). Hence the white muscle which is more active anaerobically could accumulate more inert metabolic glycogen than the red muscle (Bilinski 1969) capture handling. Nutritional status all has profound effects on the carbohydrate metabolism and blood electrolytic balance. (Mazeaud and Mazeaud 1981b. Donaldson 1981). Further. depletion of glycogen indicates the rapid utilization of energy stores to meet the energy demands warranted by the environment (Githa and Yeragi. 1998. Basha Mohideen and Sudharshan Reddy 2003). Sonawane *et al*., (2001) studied seasonal variations in tissue glycogen content in exotic fish *Cyprinuscarpio*. But studies involving carbohydrate energy reserves in fishes exposed to different nutritional media are highly scanty.

MATERIAL AND METHODS

Plane 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 shellar 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 group: age, two years .The control group of fishes were fed with control feed i.e. groundnut cake, rice bran . The experimental group of fishes was divided into two groups. Agrimin and Fishmin which are commercially available have been selected for the study. The first group of experimental fish was fed with control feed mixed with Agrimin. The second group of experimental fish was fed with control feed mixed with fishmin. The two groups of experimental fish were 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.

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).

Agrimin:
Agrimin is a product from Glaxo. 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

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 aldolase activity was estimated by the method of Bruns and Bergmayer (1963) where in the triosephosphates formed were estimated by using 2.4. dinitrophenyl hydrozine. The aldolase activity was expressed as moles of fructose 1.6 diphosphate mg/protein/hr .NAD dependent lactate dehydrogenase (LDH) activity in the direction of pyruvate formation was assayed by the method of Srikanthan and Krishna Murthy (1955). The enzyme activity was expressed in moles of formazan formed/mg/protein/hr .Succinate dehydrogenase activity was estimated by the method of Nachlas et al. (1960). The activity of SDH were expressed as M of formazan formed per mg protein per hour. Malate dehydrogenase activity was estimated by the method of Nachlas et al. (1960).The enzyme activity was expressed in moles of formazan formed 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 AND DISCUSSION

In the control. agrimin and fishmin fed fish species muscle and liver the aldolase activity levels were measured and the data was presented in table 1.

Table-1: Effect of Agrimin & Fishmin on Muscle and Liver tissue Aldolase levels of various fish species (Value expressed as moles of fructose 1. 6-diphosphate cleaved/mg Protein/hr)

Name of the Feed	Name of the parameter					
	Aldolase					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	3.612	4.824	3.926	5.059	2.967	4.054
SD	±1.21	±0.32	±0.65	±0.091	±0.25	±0.36
PC						
t						
Control Feed + Agrimin						
AV	3.643	4.992	4.051	6.734	3.123	4.262
SD	±0.96	±0.55	±0.72	±0.49	±0.74	±1.29
PC	0.85	3.48	3.18	33.10	5.25	5.13
t	*	*	*	*	*	*
Control feed + fishmin						
AV	3.620	4.975	4.11	6.546	3.320	4.176
SD	±1.24	±0.71	±0.84	±0.55	±0.72	±0.61
PC	0.22	15.1	4.68	29.39	11.89	3.00
t	*	*	*	*	*	*

Note: 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

Agrimin and fishmin fed fishes muscle and liver showed increased aldolase activity over the control feed fed ones and all the changes were found to be statistically significant (P<0.001) over control ones. The results in the control. agrimin and fishmin fed fish species muscle and liver LDH activities were measured and the data was presented in table 2. An increase in agrimin and fishmin fed fish species muscle and liver tissues was observed over the control. All the changes were found to be statistically significant (P<0.001) over their corresponding control values. Identical trends were also obtained for agrimin and fishmin fed fishes muscle and liver MDH and SDH activity levels (Table 3 and 4).

Table -2: Effect of Agrimin & Fishmin on Muscle and Liver tissue Lactate Dehydrogenase (LDH) levels of various fish species (Value expressed as moles of formazan formed/mg Protein/hr).

Name of the Feed	Name of the parameter					
	Lactate Dehydrogenase (LDH)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.159	0.248	0.174	0.282	0.137	0.196
SD	±0.004	±0.006	±0.049	±0.014	±0.063	±0.045
PC						
t						
Control Feed + Agrimin						
AV	0.179	0.286	0.246	0.574	0.153	0.242
SD	±0.002	±0.14	±0.032	±0.003	±0.072	±0.005
PC	12.57	15.32	37.93	103.54	11.67	23.46
t						
Control feed + fishmin						
AV	0.166	0.263	0.237	0.541	0.172	0.261
SD	±0.005	±0.007	±0.036	±0.077	±0.042	±0.069
PC	4.4	6.04	36.20	91.84	25.54	33.16
t						

Note: 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 Malate dehydrogenase (MDH) levels of various fish species (Value expressed as moles of formazan formed /mg Protein / hr).

Name of the Feed	Name of the parameter					
	Malate dehydrogenase (MDH)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	7.85	12.83	9.26	15.08	6.05	11.26
SD	±0.62	±0.27	±0.44	±0.82	±0.36	±1.04
PC						
t						
Control Feed + Agrimin						
AV	9.04	14.26	13.49	18.26	7.26	13.42
SD	±1.24	±0.94	±2.16	±1.24	±0.087	±2.14
PC	15.15	11.14	45.68	21.08	20.00	19.18
t						
Control feed + fishmin						
AV	8.08	13.04	10.14	16.05	7.04	12.17
SD	±1.07	±0.94	±0.072	±0.82	±0.18	±0.65
PC	28.46	16.36	9.50	6.43	16.36	8.07
t						

Note: 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-4: Effect of Agrimin & Fishmin on Muscle and Liver tissue Succinate dehydrogenase (SDH) levels of various fish species (Value expressed as moles of formazan formed /mg Protein / hr).

Name of the Feed	Name of the parameter					
	Succinate dehydrogenase (SDH)					
	Labeo rohita		*Catla catla*		*Cirrhinus mrigala*	
	Muscle	Liver	Muscle	Liver	Muscle	Liver
Control Feed						
AV	0.245	0.625	0.262	0.857	0.23	0.551
SD	±0.037	±0.064	±0.12	±0.052	±0.0036	±0.1003
PC						
t						
Control Feed + Agrimin						
AV	0.27	0.972	0.385	1.226	0.215	0.592
SD	±0.033	±0.041	±0.025	±0.064	±0.006	±0.004
PC	10.20	55.52	46.94	43.05	7.32	7.44
t						
Control feed + fishmin						
AV	0.255	0.821	0.263	0.974	0.240	0.573
SD	±0.055	±0.042	±0.01	±0.076	±0.032	±0.16
PC	4.08	31.36	0.38	13.65	3.44	3.99
t						

Note: 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.

DISCUSSION

Carbohydrate metabolism is essentially the metabolism of glucose and substances related to glucose. Glucose occupies central position of carbohydrate metabolism in an organism. representing complex groups. sequences and cycle of reactions which integrate at various points. The enzyme aldolase plays an important role in the continuation of glycolysis. The breakdown of hexoses into triores favours not only the observation of glycolysis but also synthesis of glucosal. Increased aldolase activity levels in the agrimin and fishmin fed fish tissues muscle and liver supports increased glycolysis in these tissues. This suggests increased formation of those phosphates (Phuspharani, 1997) and their mobilization for the synthesis of fatty acids and reesterification of triglycarious. An elevation in aldolase activity indicates increased oxidations of hexodes to meet the enhanced energy demand during growth of the fishes. This also indicates activity channeled to glycolysis (Geethabali and Chandra Sekhar, 1988. Pushparani, 1997).

Lactate and Pyruvate contents constitute the inter convertible products of glycolysis their interconversion is mediated by the enzyme LDH which requires NAD^+/ $NADH^+$ as coenzymes. The NAD^+ - dependent LDH catalyze the conversion of lactate. Increase in the agrimin and fishmin fed fishes muscle and liver tissues (Table-2) supports increased conversion of lactate to pyruvate or vice versa. in general variations in the activity level of LDH indicates differences in the physiological and metabolic status of the respective tissues.

SDH and MDH enzymes are oxidative enzymes of TCA Cycle. SDH present in mitochondria and cytoplasm requires NAD as the coenzymes and both these enzymes contribute to the synthesis of ATP. Therefore these enzymes also have been taken up for investigation in the present study. Agrimin and fishmin enhanced the various fish species muscle and liver tissues SDH and MDH activity levels (Table-3.4). Elevated levels of SDH and MDH in agrimin and fishmin fed fish tissues evidently demonstrate elevated rate of Krebs cycle activities. This could be an indication of enhancement in oxidation phosphorylation towards ATP synthesis and has a biochemical background presumably favourable for the generations of ATP.

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