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# Impact Of Agrimin And Fishmin On The Aspects Of Carbohydrate Metabolism & Tca Cycle Enzymes In The Muscle & Liver Tissue Of The Fish Species, *H.Molitrix, C.Carpio, C.Idella*

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***Abstract:*** *The present study is focused on the nutritional impact of agrimin and fishmin on the aspects of carbohydrate metabolism and TCA cycle enzymes in the muscle and liver tissues of the selected fish species. The fishes are divided into three groups treated as control, control and agrimin, control and fishmin and fed with selected nutrients respectively and the parameters evolved were total glucose, blood Glucose, Glycogen, Aldolase, Lactate dehydrogenase (LDH), malate dehydrogenase (MDH), succinate Dehydrogenase (SDH). Supplementary feeds caused an increase in the levels of total carbohydrates, glycogen and blood glucose in the muscle and liver of the experimental fish and the changes in all the above parameters were found to be statistically significant ( P<0.001) over control feed fed fish muscle and liver. More percent changes were found to be induced by Agrimin as compared to fishmin.*

***Key word:*** *Agrimin, fishmin, Carbohydrates, lactate dehydrogenase, malate dehydrogenase.*

## I. 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. Among the industrial processes based on carbohydrates may be mentioned textiles, paper,

plastics, explosives, fermination industries and alcohol. The vitamin, ascorbic acid, is a carbohydrate derivative and some of the plasma expanders belong to the class of carbohydrates. Glycosides of the digitalis-strophanthus group are used in medicine and it is recognized that the invasiveness and antigenic nature of some bacteria is due to their polysaccharide envelope. A great many of the wondrous colours of the plant kingdom are due to the glycoside pigments of the anthocyanin and flavones groups. From the theoretical standpoint, the genesis of carbohydrates in plants through the mechanism of photosynthesis, the complicated steps in the enzymatic breakdown of the simple sugar glucose to carbon di oxide and water, and the phenomena of muscular contraction and bacterial fermentation have fascinated many generations of biochemists. Inside the animal cell, the storage form of energy is present in the form of glycogen. The ubiquitous nucleic acids, deoxyribonucleic acid in the nucleus and ribonucleic acid in the cytoplasm and nucleolus, derive their name from the carbohydrate present in them. The nourishment of the cell come from the circulating glucose of the extra cellular environment and the activities of the cell may be profoundly altered by the mucopolysaccharides of the surrounding extra cellular tissue or the blood borne glycoprotein hormones of the anterior pituitary. Mucopolysaccharides are also biological lubricants in the form of saliva, mucus and synovial fluid. (Prakash, Arora, 1998)

The term carbohydrate originated in the belief the naturally occurring compounds of this class could be represented by a general formula C ($H_2O$). Though formaldehyde, acetic acid, and lactic acid fulfill this formula requirement, they are not carbohydrates and some of the methylpentoses and other "deoxy" sugars which belong to the Carbohydrate class would be excluded by this requirement. The term carbohydrate is retained, therefore, as a matter of convenience rather than of precise definition. The term carbohydrates now encompasses a variety of substances which include polyhydroxy aldehydes and ketones, alcohols, acids, amino sugars and their derivatives as well as the numerous products formed by the condensation of these different types of compounds with each other by means of glycosidic linkages to form oligosaccharides and polysaccharides. Polyol (glycerol and ribitol) phosphate ployers like teichoic acids are also included in this class of compounds. The elucidation of their chemistry has been a saga of great effort in organic chemistry and even today presents an area of challenge and great biological significance. The structures of eight aldohexoses were carefully elucidated which only three occur naturally while the rest were supplemenrtary. Now over two hundred different monosaccharides are known to be present in nature. Many Amino sugars, branched chain sugars and a wide variety of N-acetyl N-glycolyl neuraminic acids (Sialic acids) have been isolated. Several antibodies like streptomycin, puromycin, neomycin and amino-glycoside antibodies like kanamycin certain unusual types of sugar residues.

Carbohydrates may be broadly classified into three main groups:

1. Monosaccharides, e.g., ribose, glucose, glactose, sedoheptulose.
2. Oligosaccharides with 2-10 monosaccharide units e.g., sucrose, lactose.

3. Polysaccharides e.g., glycogen, starch, cellulose, insulin.

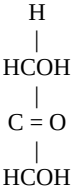
Owing to their sweet taste and crystalline character the mono, di and trisaccharides are generally grouped together under the name of sugars. The polysaccharides are tasteless, amorphous and sparingly soluble substances, which on hydrolysis yield monosaccharides.

**1.2 Classification:**

Carbohydrates, or Saccharides are polyhydroxyaldehydes or polyhydroxyketones and their derivative. The simplest of these, the monosaccharides, may be considered to be hydrates of carbon ($CH_2O$), for which is 3 to 8. Oligosaccharides are carbohydrates comprised of a multiple (2 to 10) of monosaccharides joined by acetal or ketal linkages. Polymers of saccharides exceeding 10 in number are referred to as polysaccharides; the chains of monosaccharide units in these molecules may be linear or branched.

The carbon chain of a monosaccharide is usually unbranched. Each carbon atom, except one, carries a -OH group ; the remaining carbon is a carbonyl group. If the carbonyl group is at a chain terminus, the monosaccharides is designated as an aldose;


If the C=O is internal in the chain, the sugar is a ketone derivative and is called a ketose:




The generic names given to monosachharides identify both the number of carbons and the nature of the carbonyl group. As examples, glyceraldehydes is an aldotriose; dihydroxyacetone is a ketoraiose, ribose is an aldopentose; glucose is an aldohexose; and fructose is a ketohexose.

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 (Fakuda, 1958) 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 co-enzymes. 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 glyceral 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).

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 have 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 *Cyprinus carpio*. But, studies involving carbohydrate energy reserves in fishes exposed to different nutritional media are highly scanty.

## II. Materials and Methods

**2.1 Estimation of Total Carbohydrates**

The Carbohydrate content was estimated by the method of Carrol et al., (1956). The tissues 0.2 ml homogenates were individually homogenized in 10% trichloroacetic acid and centrifuged at 3000 rpm for 15 minutes. To 1.0 ml of TCA

supernatant, 4 ml of anthrone reagent was added and the colour was read against the reagent blank at 600 nm in a spectrophotometer. From the optical density, the total carbohydrate content was calculated in comparison with the standard and the values were expressed as mg carbohydrates/gm/wet weight of the tissue.

**2.2 Estimation of blood Glucose**

Glucose in the samples was determined by colorimetric method as escribed by Nelson and Somogyi (1952). 0.1 ml of blood was collected. 3.9 ml of deproteinizing solution (5% Zinc sulphate and 0.3 N Sodium hydroxide in 1:1 ratio) was added to it and the mixture was centrifuged at 3000 rpm for 10 minutes. To 1 ml of the supernatant from each of these mixtures, 1 ml of alkaline copper reagent was added and it was shaken vigorously and heated in a boiling water bath exactly for 20 minutes. Then it was cooled and 1 ml of arsenomolybdate colour reagent was added to it. Distilled water was added to the entire solution till it measured 10 ml and the optical density of the colour developed was measured in a spectrophotometer at a wave length of 540 nm. A blank and glucose standards were also run simultaneously. Glucose content was expressed as mg of glucose / 100 ml of blood.

**2.3Estimation of Glycogen**

Glycogen content in liver and muscle of fish was estimated using the anthrone reagent method as described by carroll et al., (1956). Since glycogen concentration in muscle is known to vary in different regions of body (Amano et al. 1953, Fraser et al., 1966) care was taken in dissecting out this sample from the same region of body of fishes i.e., The antero dorso lateral region of the trunk. The organs were digested with 3.0 ml of hot 30% potassium hydroxide (Hassid and Abraham, 1957). The digestate was cooled and 3.75 ml of absolute ethanol was added to it. The entire mixture was kept over night in a refrigerator. Then the mixture was centrifuged for 15 minutes at 2500 rpm. Decanted The supertanant and 10.0 ml of warm distilled water was added to the residue to dissolve the precipitated glycogen. To 0.2 ml of this 1.8 ml at distilled water and 0.5 ml of 2% anthrone reagent dissolved in 72% concentrated sulphuric acid were added and heated in a boiling water bath exactly for 10 minutes. The mixture was cooled and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 620 nm. A blank and glucose standards were also run similarly. The glycogen content is expressed as mg/g wet wt of the organ.

**2.4 Estimation of Aldolase Activity (Fructose1, 6-Diphosphate D-Glyceraldehyde-3-Phosphate Lyase: Ec. 41.2.6)**

The aldolase activity was estimated by the method of Brons and Bergmayer (1963) where in the triosephosphates formed were estimated by using 2,4, dinitrophenyl hydrozine. 2% (w/v) homogenates of tissues were prepared in cold distilled water and centrifuged to 1000 rpm for 5 min. The reaction mixture of 3 ml contained 1.75 ml of collidine hydrozine buffer (pH 7.4) 0.25 ml of fructose-1-6 diphosphate (0.02 M pH 7.4) 0.5 ml of distilled water and 0.5 ml of homogenate supernatant the reaction mixture was incubated for 15 min at $37^0$C and the reaction was arrested by adding 3.0 ml of 10% TCA. Then the contents were filtered 1.0 ml of 0.75 N-NaOH was added to 1.0ml of the filtrate and allowed to stand for 10 min at room temperature. Then 1.0 ml of 2.4ml, dinitrophenylhydrazine was added and the contents were incubated at $37^0$C for 10 min. After incubation, 8 ml of 0.75 N NaOH was added. The reddish brown colour developed was read at 540 nm in a spectrophotometer against a zero time control.The aldolase activity was calculated according to Bruns (1954) and the values were expressed as moles of fructose 1,6 diphosphate mg/protein/hr .

**2.5 Determination of Lactate dehydrogenase (LDH) (L- Lactate NAD with Oxidoreductase EC: 1.1.1.27)**

NAD dependent lactate dehydrogenase activity in the direction of pyruvate formation was assayed by the method of Srikanthan and Krishna Murthy (1955).5% homogenates of the tissues were prepared in ice cold 0.25 M sucrose solution, and 0.5 ml of homogenates was used. The homogenates were centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2.0 ml contained 4 moles of lithium lactate 100 moles of potassium phosphate buffer pH 7.4, 0.1 moles of NAD and 4 moles of INT (2, 4-iodophenyl-1, 3-(4-nitrophenyl)-5-phenyl tetrazolium chloride). The reaction was initiated by the addition of 0.5 ml of supernatant. The reaction was arrested by adding 5.0 ml of glacial acetic acid. The iodoformazan formed was extracted overnight in 5 ml of toluene at $5^0$C. The optical density of the colour was measured in spectrophotometer at 495 nm against toluene blank. The enzyme activity was expressed in moles of formazan formed/mg/protein/hr.

**2.6 Assay of malate dehydrogenase (MDH) (L-malate :NADwith oxidoreductase; EC.1.1.1.37) activity.**

Malate dehydrogenase activity was estimated by the method of Nachlas *et al.,* (1960). 5% homogenate of tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000 rpm for 15 min. The supernatant were used for the assay. The reaction mixture of 2.5ml contained; 60 moles of sodium malate, 100 moles of potassium phosphate buffer (pH 7.0) 4 moles of nicotinamide adinine dinucleotide (NAD) 40 moles of INT and 0.5 ml of the tissue homogenate supernatant. The other steps followed are the same as described for SDH.The enzyme activity was expressed in moles of formazan formed mg protein /hr.

**2.7 Measurement of succinate Dehydrogenase (SDH) (Succinate acceptor : Oxidoreductase EC : 1.3.99.1) activity**

Succinate dehydrogenase activity was estimated by the method of Nachlas *et al.,* (1960). 5% homogenates of the tissue were prepared in 0.25 M ice cold sucrose solution and 0.5 ml was used. The homogenates were centrifuged at 3000 rpm for 15 minutes. The supernatant fraction was used for the enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 moles of sodium succinate, 100 moles of phosphate buffer (pH 7.4) and 2 moles of INT (2, 4-iodophenyl-1-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride. The reaction was initiated by the addition of 0.5ml of the tissue extract and incubated for 30 minutes at 320C. The reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold into 5 ml of toluene. The intensity of the colour developed was read at 495 nm

against blank. The activity of SDH were expressed as M of formazan formed per mg protein per hour .

## III. Results

The agrimin and fishmin fed fish species muscle and liver showed enhanced levels of their total carbohydrate content over their corresponding control values and the increment was found to be statistically significant ($P<0.001$) over the control values. Liver appeared to possess more carbohydrate content compared to the muscle tissue .So does the case of glycogen.

Statistically significant ($P<0.001$) increase in the liver and muscle and liver tissues glycogen content over the control feed fed fish tissues was registered in the present study for the fish tissues fed with agrimin and fishmin. Liver tissue of the three fish species appeared to possess high glycogen content in the present study. (Agrimin and fishmin fed fishes serum showed increased levels of their glucose content and the changes were found to be statistically significant ($P<0.001$) over their control values.

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

## IV. 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 reactions concerned with metabolism of lipids and proteins as these molecules serve the source of carbon in the synthesis of cellular components (Nelson and Cox 2000). Glycogen is the chief carbohydrate present in tissues, while glucose is of the blood and other body fluids. Glycogen a storage carbohydrate from intestinal absorption is inadequate. Glycogen breakdown into glucose is governed by the extrinsic and intrinsic factors which also controls the physiology of an organism.

Carbohydrate metabolism essentially constitutes two segments namely synthesis of Carbohydrates which includes gluconeogenesis and catabolism which includes glycolysis, glycogenolysis, pentose pathway and Krebs cycle.

The catabolic pathways not only fulfill the needs of energy demands but also supply the amphibolic intermediates and reduced neuotides (NADPH) required for protein and lipid metabolism (Nelson and Cox, 2000).

The mechanism by which glycogen is synthesized and broken down in tissues is initiated by phosphorylase enzymes. The process of glycolysis in tissues commences with the consent of glycogen breakdown and the glucose released is fragmented into three carbon compounds, pyruvic acid and lactic acid by a series of enzymes under anaerobic conditions. The end products lactate and pyruvate are interconvertible by the enzyme lactate dehydrogenese (LDH). Pryuvate undergoes oxidative de carboxylation by pyruvate dehydrogenese to provide acetyl Co-A.

Acetyl-Co-A is an essential substrate for Kreb's cycle, which generates reduced nucleotides for the ultimate generation of ATP molecules through electron transport. Amphibiolic intermediates formed in the Krebs cycle may be channeled into amino acid or fatty acid synthesis. Channeling of carbohydrate precursors into energy yielding reactions or supplemenrtary reactions depends on the biochemical make up of an organ system concerned or physiological alterations dependant on changed environmental conditions. Pyruvate utilisation for energy requirements is tissue specific and varies according to environmental conditions imposed on the animal.

Since biological system has some flexibility, animals use this capacity to divert metabolic pathways to an alternate source to synthesize energy to overcome the energy crisis created by the stress.

The increased levels of total carbohydrates, glycogen in the muscle and liver of agrimin and fishmin fed fishes indicate that these tissues and blood show an upsurge in glycogenesis and glycogenolysis and this situation was more in the tissues and blood of agrimin and fishmin fed fishes. This trend further

may be due to accumulation of feeding capacity of the fish species selected for the study. The overall results on carbohydrates glycogen and glucose in the present study supports an upsurge of carbohydrate metabolism in the fish tissues and blood fed on either agrimin or fishmin.

Aldolase plays an important role in the continuation of glycolysis at the level of FDP. The breakdown of hexoses into triores favour 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 (Phospharani, 1997) and their mobilisation for the synthesis of fatty acids and resterification of triglycarious (Muller and Seitz, 1984). 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 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 NDH 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. 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.

**Table: 1** Effect of Agrimin & Fishmin on total Carbohydrates content of the liver & muscle tissue of the fish species *H.molitrix, C.carpio, C.idella* (Value expressed as mg/gm wet wt. of tissues)

| | ***Cyprinus carpio*** | | ***Hypophthalmichth ys molitrix*** | | ***Ctenopharyngodo n idella*** | |
|---|---|---|---|---|---|---|
| | **Muscle** | **Liver** | **Muscle** | **Liver** | **Muscle** | **Liver** |
| **Name of the Feed** | | | | | | |
| Control Feed | | | | | | |
| AV | 10.52 | 24.67 | 12.11 | 26.08 | 9.67 | 20.36 |
| SD | ±0.729 | ±1.68 | ±0.89 | ±1.24 | ±0.74 | ±2.30 |
| PC | | | | | | |
| t | | | | | | |
| Control Feed + Agrimin | | | | | | |
| AV | 15.08 | 30.22 | 16.75 | 31.62 | 11.08 | 26.45 |
| SD | ±0.52 | ±0.66 | ±0.72 | ±0.84 | ±1.10 | ±0.96 |
| PC | 0.62 | 0.89 | 1.14 | 0.26 | 0.34 | 1.26 |
| t | * | * | * | * | * | * |
| Control feed + fishmin | | | | | | |
| AV | 13.11 | 22.52 | 14.69 | 29.78 | 12.49 | 22.52 |
| SD | ±0.44 | ±0.52 | ±0.36 | ±2.12 | ±0.37 | ±0.22 |
| PC | | | | | | |
| 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

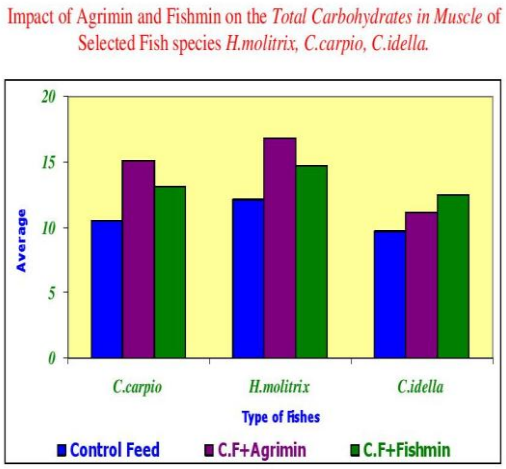



**Fig:1** Effect on Total Carbohydrates in Muscle

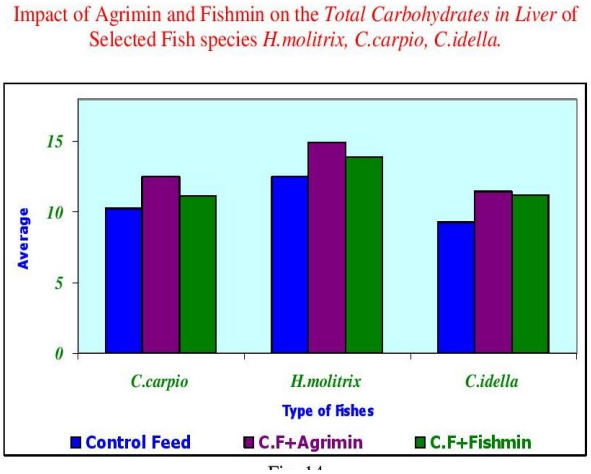



**Fig: 2** Effect on Total Carbohydrates in Liver

**Table: 2** Effect of Agrimin & Fishmin on the blood Glucose level of *H.molitrix, C.carpio, C.idella* (Values expressed as mg of Glucose / 100ml of blood)

| | **Name of the Parameter** | | |
|---|---|---|---|
| | **Blood Glucose** | | |
| **Name of the Feed** | ***Cyprinus carpio*** | ***Hypophthalmichth ys molitrix*** | ***Ctenopharyngodon idella*** |
| Control Feed | | | |
| AV | 105.27 | 108.19 | 109.15 |
| SD | □ 1.24 | □ 3.16 | □ 2.14 |
| PC | | | |
| t | | | |
| Control Feed + Agrimin | | | |
| AV | 114.45 | 118.37 | 119.96 |
| SD | □ 3.16 | □ 0.94 | □ 2.99 |
| PC | 8.72 | 9.40 | 9.90 |
| t | * | * | * |
| Control feed +Fishmin | | | |
| | | | |
| AV | 110.27 | 110.91 | 114.52 |
| SD | □ 1.24 | □ 2.16 | □ 4.13 |
| PC | 4.74 | 2.51 | 4.91 |
| 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

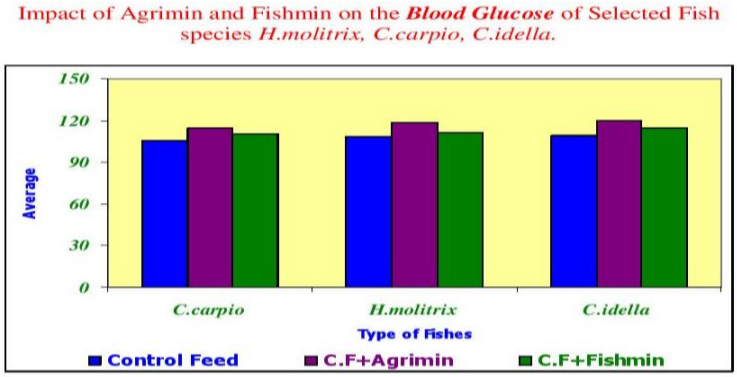



**Fig: 3 Effect on Blood glucose**

**Table: 3** Effect of Agrimin & Fishmin on Muscle and Liver tissue Glycogen levels of *H.molitrix, C.carpio, C.idella* (Value expressed as mg glycogen/g wet wt. of tissue)

| Name of the Feed | Name of the Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Glycogen | | | | | |
| | *Cyprinus carpio* | | *Hypophthalmichth ys molitrix* | | *Ctenopharyngodo n idella* | |
| | **Muscle** | **Liver** | **Muscle** | **Liver** | **Muscle** | **Liver** |
| Control Feed | | | | | | |
| AV | 2.29 | 11.56 | 2.54 | 12.72 | 2.43 | 12.10 |
| SD | ±0.055 | ±0.34 | ±0.051 | ±1.02 | ±0.67 | ±1.25 |
| PC | | | | | | |
| t | | | | | | |
| Control Feed + Agrimin | | | | | | |
| AV | 2.40 | 12.72 | 2.87 | 14.05 | 2.74 | 13.36 |
| SD | ±0.055 | ±0.75 | ±0.025 | ±1.49 | ±0.074 | ±1.08 |
| PC | 4.80 | 10.03 | 12.99 | 10.45 | 12.75 | 10.41 |
| t | * | * | * | * | * | * |
| Control feed +Fishmin | | | | | | |
| AV | 2.33 | 12.45 | 2.60 | 13.41 | 2.56 | 12.72 |
| SD | ±0.074 | ±0.77 | ±0.15 | ±1.67 | ±0.027 | ±1.45 |
| PC | 1.74 | 7.6 | 2.36 | 5.42 | 5.34 | 5.12 |
| 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

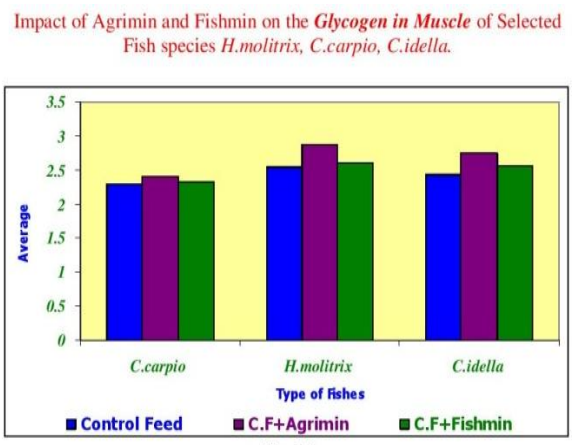



**Fig: 4** Effects on Total Glycogen in Muscle

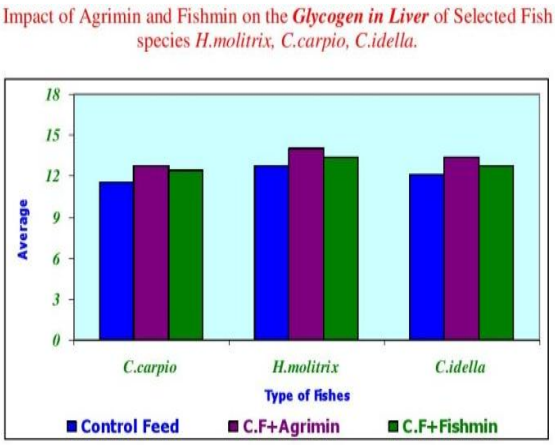



**Fig: 5** Effects on Total Glycogen in Liver

**Table: 4** Effect of Agrimin & Fishmin on Muscle and Liver tissue Aldolase levels of *H.molitrix, C.carpio, C.idella* (Value expressed as moles of fructose 1, 6-diphosphate cleaved/mg Protein/hr)

| Name of the Feed | Name of the Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Aldolase | | | | | |
| | *Cyprinus carpio* | | *Hypophthalmichth ys molitrix* | | *Ctenopharyngodo n idella* | |
| | **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 | * | * | * | * | * | * |

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 ***Aldolase in Muscle*** of Selected Fish species *H.molitrix, C.carpio, C.idella.*

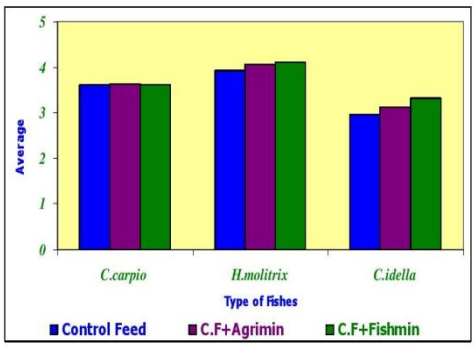



**Fig: 6** Effects on Total Aldolase in Muscle

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

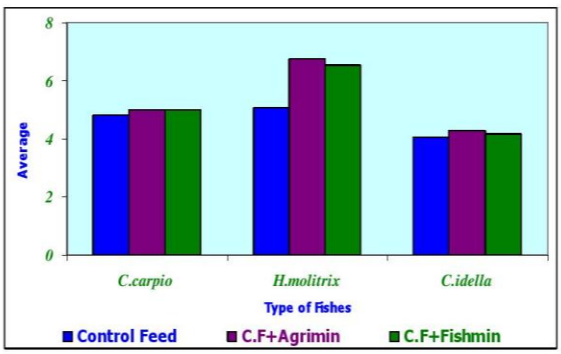



**Fig: 7** Effects on Total Aldolase in Liver

**Table: 5** 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) | | | | | |
| | *Cyprinus carpio* | | *Hypophthalmichth ys molitrix* | | *Ctenopharyngodo n idella* | |
| | **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 | | | | | | |

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

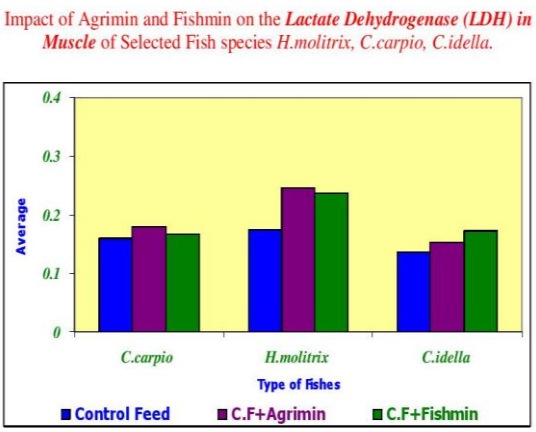



**Fig: 8** Effects on Total Lactate Dehydrogenase in Muscle

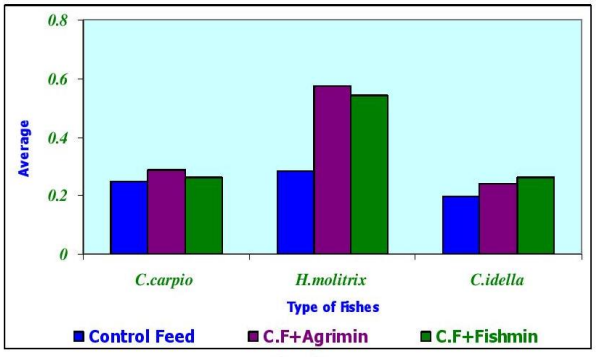



**Fig: 9** Effects on Total Lactate Dehydrogenase in Liver

**Table: 6** Effect of Agrimin & Fishmin on Muscle and Liver tissue Malatedehydrogenase (MDH) levels of *H.molitrix, C.carpio, C.idella* (Value expressed as moles of formazan formed /mg Protein / hr)

| Name of the Feed | Name of the Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Malatedehydrogenase (MDH) | | | | | |
| | *Cyprinus carpio* | | *Hypophthalmichth ys molitrix* | | *Ctenopharyngodo n idella* | |
| | **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 | | | | | | |

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

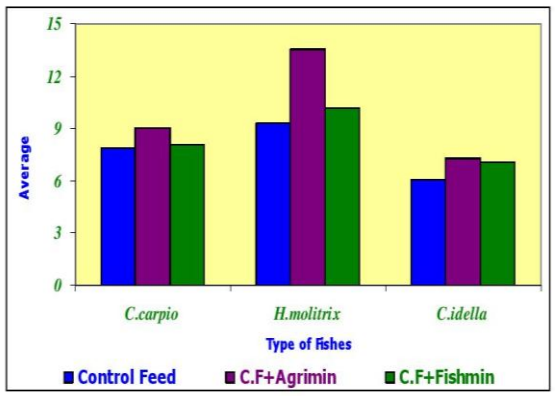



**Fig: 10** Effects on Total Malate Dehydrogenase in Muscle

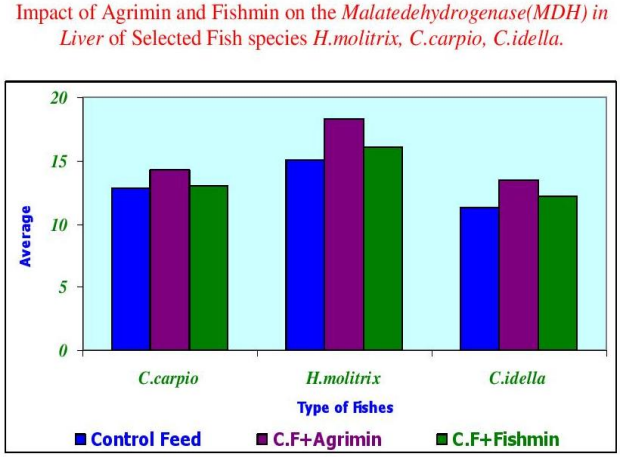



**Fig: 11** Effects on Total Malate Dehydrogenase in Liver

**Table: 7** Effect of Agrimin & Fishmin on Muscle and Liver tissue Sucinate dehydrogenase (SDH) levels of *H.molitrix, C.carpio, C.idella*(Value expressed as moles of formazan formed /mg Protein / hr)

| Name of the Feed | Name of the Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Sucinated dehydrogenase (SDH) | | | | | |
| | *Cyprinus carpio* | | *Hypophthalmichth ys molitrix* | | *Ctenopharyngodo n idella* | |
| | **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 | | | | | | |

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

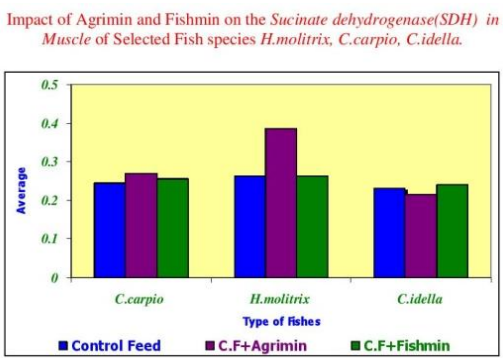



**Fig: 12** Effects on Total Sucinate Dehydrogenase in Muscle

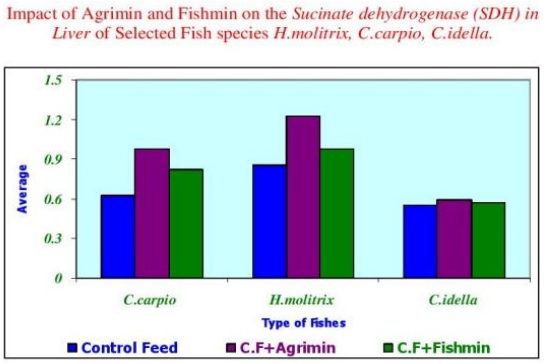



**Fig: 13** Effects on Total Malate Dehydrogenase in Liver

## V. Conclusion

In the muscle and liver of supplementary feeds fed fish species the parameters of carbohydrates metabolism were studied. Supplementary feeds caused an increase in the levels of total carbohydrates, glycogen and blood glucose in the muscle and liver of the experimental fish and the changes in all the above parameters were found to be statistically significant ( P<0.001) over control feed fed fish muscle and liver. More percent changes were found to be induced by Agrimin as compared to fishmin. This is found more in C.carpio followed by H.molitrix and C.idella. The key enzymes involved in Carbohydrate metabolism like aldolase, LDH, SDH, MDH showed an increasing trends in the Agrimin fed fishes muscle and liver than fishmin, and the changes were found to be statistically significant over the control ones. More percent increase in all the above enzymatic activities C.carpio followed by H.molitrix and C.idella. The increase in total carbohydrate and glycogen content in the fish species muscle and liver indicates an upsurge in glycogenesis and glyconeogenesis. This is further supported by an increase in the activity of aldolase activity under supplementary feeds stress. Increased LDH activity supported increased rates of conversion of Lactate to pyruvate.The feed has further enhanced the oxidative enzymatic activities like SDH & MDH in the muscle and liver and this is indicative of prevalence of increase of oxidative metabolism in the muscle and liver of selected fish species under supplementary feeds stress.

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