A comparative study for some aspects of energy metabolism in two samples of intestinal cestodes: in house frog and domestic pigeon

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Abstract

Two intestinal cestodes were chosen to make a compares, considering some aspects of energy metabolism; *Ophiotaenia bofonis* habitat small intestine of the house frog *Bufo viridis viridis*, and *Cotugnea columbae* habitat small intestine of the domestic pigeon *Columba livia domestica*.

The present study reviled that concentration of macrobiomolecules , the energy raw materials, in *O. bofonis* is higher than that of *C. columbae*. There were significant differences at $p \leq 0.05$ between the concentration of total carbohydrates, protiens and lipides: (240, 181 µg/ml and 196mg/100ml, respectevely in the pigeon cestode and 105, 153 µg/ml and 133mg/100ml, respectively in the frog cestode).

High performance liquid chromatography technique (HPLC) analysis for fatty acid elucidated that each of the two helminthes contain six types of fatty acid. *O. bofonis* found to have three saturated fatty acids: stearic, palmatic and myristic, 2.72%, 2.29% and 0.89% respectively and three unsaturated fatty acid: linoleic, linolienic and oliec, 73.13%, 13.07% and 7.91%, respectively. On the other hands, *C. columbae* found to have four saturated fatty acid: stearic, palmatic, myristic and caproic, 51.43%, 45.89%, 2.38% and 0.19% respectively, and two unsaturated fatty acides: linoleic, linolienic, 0.09% and 0.06%, respectively.

Enzymatic study pointed to the significant differences at $p \leq 0.05$ between the enzymes activity in frog cestode and that of pigeon cestode. Activity of G6PDH and LDH (cytosolic enzymes) and SDH and FR (Mitochondrial respiretory enzymes), were :7.514 U/L, 56 IU/L, 130 nm FAD/min/ mg protien and 198 nm NADH/min/mg protien in *O. bofonis* extract; and 7.514 U/L, 56 IU/L, 130 nm FAD/min/ mg protien and 198 nm NADH/min/mg protien in *C. columbae* extract.

The prevous resultes may reffered to a relativly higher activity of the enzymes under study, those take a part in glycolisis and energy metabolism libration in *O. bofonis* than that in *C. columbia,* in spite of that the occumulated souces of energy were more aboundant in pigeon cestode.

Key words: energy metabolism, *Ophiotaenia bofonis*, *Cotugnea columbae*, carbohydrates, lipids, fatty acids, G-6-PD, LDH, SDH, FR.

Introduction

Parasites are in need for unusually great demand on energy generating mechanisms. So, too, does the need to produce vast numbers of offspring. Protection against the immune system of the host is another costely energy drain (Bryant and Behm, 1989).

There is no doubt that the variance in parasites demand for energy is closely related to host type, habitat (location in the host body), in addition to the echosystem in which the host themselves lieve (Chapple, 1980). Intestinal cestodes are a group of endoparasitic helminthes. Their elongated body enables them to live in the tubular habitat (Smyth and McManus, 2007). Cestodes miss alimentary canal, thus derive their nutrition from the host`s gut across it`s highly specialized, metabolically active body cerface or tegument (Smyth and McManus, 2007). The metabolic and *in vitro* studies suggest that there is a complex nutritional relationship occurs between the gastrointestinal cestodes and it`s host (Sonune, 2012).

The most important variation in the enveronment arround the parasite is the avaibility of food and oxygen. As for parasitic flatworms, they are generally do not use oxygen as the final electron acceptor, but use fermentative processes to obtain most of their ATP. Most adult flatworms inside the final host produce end products for the fermentative carbohydrate breakdown, such as succinate, acetate, propanioate and lactate.These end products are formed via malate dismutation, a fermentative pathway which is present in all types of parasitic cestodes and nematodes (Maule and Marks, 2006) . Thus,the energy metabolism of parasitic flatworms is clearly and substencially different from that of their hosts. Apparently, they have adapted to exploid anaerobic niches and the surplus substrate that are commonly present in their enveronment. This enable them to use low-effeciency catabolic pathways, and lose several anabolic pathways, such as those involved in the biosynthesis of fatty acids and steroles. Obveously, parasitic flatworms adapted to an apportunistic way of life (Bryant and Behm, 1989)

The choosen cestodes of the present study were *Ophiotaenia bofonis*; class: cestoda (Eucestoda); order: proteocephalidea; family: proteocephalidae. Subfamily: Proteocephalinae. *Ophiotania bofonis* habitat small intestine of house frog (*Bufo viridis viridis*). The other cestod was *Cotugnia columbae*; class: cestoda; order: cyclophyllidea; family: davaineidae. *Cotugngnia columbae* habitat small intestine of domestic pigeon (*Columba livia*) (Yamaguti,1959 and Jones & Bray, 1994).

Researches about energy metabolism in *Ophiotaenia spp*. and *Cotugnia spp*. are scarce. Some studies were epidimeological (Silva , *et al.* ,2006; Begum and sehrin, 2012 and Ribeir, *et al.* 2014), otheres were taxonomic

(Chambrier, *et a*l., 2012; shukla, *et al*, 2012; Gamil, 2012 and Sanap and Siddiqui, 2013), the otheres were biochemical (Al-Naftachi, 2006; Waghmare and Chavan, 2010; and Biswal, *et al*.,2012).

Therefore, this research aimed to compare betweenthe some aspects of energy metabolism of *Ophiotaenia bofonis* as an amphebian intestinal cestode and *Cotugngnia columbae* as avian intestinal cestodes; trying to give some concepts about the effects of host ecosystem variation on the energy metabolism of their intestinal cestodes.

Materials and Methods

1-Specimen collection: The adult cestodes *Ophiotaenia bofonis* were collected from the upper part of the small intestine of *Bufo viridis viridis* (the house frog) , which were obtained from different areas of Nenivah Governarate, whereas the adult cestodes *Cotugnia columbae* were collected from small intestine of *Columba livia domestica* (domestic pigeon) which were obtained from local markets in Mosule City. The collection were made during 2011- 2012. The collected animals then bringed and dissected in the research lab/Biology Department/College of Science/ University of Mosul.

The collected cestodes were washed several times with NaCl buffered seline 0.85%, put in petridish, examined under Hamilton dissecting microscope. wet wieght for each sample was fixed. Then kept in a deep freeze at -18ºC for subseqent studies. Some helminthes were squized and fixed in hot formalin 10%, then dehydrated with gradiant ethanol concentrations, clarified by lactophenol and mounted by DPX. Classification was performed in Biology Department, College of Science, University of Mosul, depending on Yamaguti (1959) ; Schmidt (1986); Jones and Bray (1994); Scholz and Chambrier (2003).

Biochemical studies:

I-Preparation of worms extract: The worms were suspended in a 0.05M Tris-HCl buffer (7.4) at a concentration of 10% wet wight/volume; then the suspension homogenized in a tissue grinder. Cell membranes disrupted using ultrasonic disintegration (MSE), a 12000 vibration/second for 30 second in ice-bath. Four cycles of sonication were applied with the suspention. Ultracentrifugation at 15000g/30minutes was done using MSE superspeed ultracentrifuge. The supernatent fraction was choosen for the accessive biochemical studies (Al-Naftachi, 2006).

II- Preparation of mitochondrial extract: As for the mitochondrial enzymes, succinate dehydrogenase and fumarate reductase, the worms were extracted utilizing method adopted by Pumpori and Srivastava (1987). A modified mitochondrial medium (pH 7.4) described by Scheibel, *et al.* (1968) was used. The medium consisted of: 0.24M sucrose, 0.15% bovine serum albumine (BSA) and 0.00M ethylendiaminetetraacetic acid (EDTA). Worm`s tissue were granded in a concentration of 10% (W/V), the homogenate then cenrifuged at 1000g for 20 minute, the supernatent then recentrifuged at 15000g for 30 miute. The precepetate which contain mitochondrial pellet was washed twice with modified mitochondrial medium. Washed pellet were suspended finally in 0.05 M Tris-HCl buffer pH 7.4. The mitochondrial fraction was desrupted by four successive barests 30 second (with 1 minute cooling intervals) using

ultrasonic disentegrator, all the above operations wre carried out at 4ºC.

III-Estimation of macromolecules:

A-Estimation of total carbohydrates: Herbert, *et al*. method (1971) was applied to estimate cestodes carbohydrates in the worms extract. Absorbance was mesured at 488nm. Carbohydrate concentration were estimated depending on carbohydrate standered curve.

B-Estimation of total protiens: Lowry, *et al*. colorometric method (1951) was used to estimate protien concentration in the worms extract. Foline reagent react with protein to produce blue complex in alkaline medium. Absorbance was mesured at 750nm. Concentartion of protien was estimated using protein standered curve.

C-Estimation of total lipids: Chabrol and Chardonnet colorometric method (1937) was used to evaluate whole lipids content (cited by Tietz, 1980). 20 µL of worms extract was heated with concentrated sulphoric acid. Phosphovaniline indicator was added to the mixture to produce purple-red color complex. Absorbance were determined at 540nm. Whole lipids in each sample were estimated depending on the rule:

$$\text{Whole lipids concentration mg/100cm} = \frac{\text{sample absorbance} - \text{blank absorbance}}{\text{standered absorbance} - \text{blank absorbance}} \times 500$$

IV-Fatty acids analysis:

A-Fatty acids extraction: Worms extract was lyophilized using Christ-BETA-Liophilizer/Germany. Fatty acids were extracted according to Al-Kaisy, *et al*. modified method (1991). 200mg of dried extract wre dissolved in 10ml of 7.5N NaOH (which prepared in 60% methnol). The mixture then heated to 105ºC for 90minutes, after cooling, 12ml of distilled water added, the mixture then acidified using 20% sulphoric acid to adjustified pH to 2. Fatty acid mixture withdrawn with saperating funnel after mixing with diethyl ether. Rotary vacum evaporater was used to extract fatty acid mixture.

B-HPLC analysis for fatty acids: High performance liquid chromatographic technique (HPLC)-reversable type was used to analyze fatty acid mixture.

1ml of acetyl chloride was added to the crude fatty acids that prepared before (Al-Kaisy, *et al*. 1991). Fatty cid esters and authentic fatty acids were analysed using the following conditions:

C18 colum (4.6×250)mm, mobile phace: (Acetonitrile 60% +water 40%), flow rate: 0.4 ml/minute, wave length: 254nm (adopted by Elliot, *et al*. 1989 and Al-mowla, 2010). Analysis of worms fatty acids and authentic fatty acids was performed in Ibn-Sena Company Labs/Bagdad University.

V-Enzymes studies:

A-Assay system for Glucose-6-phosphate dehydrogenase (G6PDH) activity:G6PDH (EC1.1.1.49) is a cytosolic enzyme that catalyze the first step in the pentose phosphate shunt, oxidizing G-6-P to 6-P-gluconate and reduce NADP to NADPH. The activity of this enzyme is mesured using Tietz spectrophotometric modified method (1995). Formation rate of NADPH is proportional to the G-6-P activity,which is mesured spectroscopically as an increase in absorbance at 340nm. Unit of enzyme activity in the worms extract was estimated according to the following rule:

$$\text{G-6-PD(IU/L)} = \frac{\Delta\,\text{absorbance}}{\text{minutes}} \times 492$$

B-Assay system for Lactate dehydrogenase (LDH) activity: LDH (EC1.1.1.27) activity were evaluated depending on a method adopted by wolf (1989). LDH reduce pyruvate to lactate in the presence of NADH (co-factor).

A specific analysis set, provided by Biomerierux/France was used. Estimation of LDH activity depends on the reaction betweenthe residual pyruvate and 2,4-Dinitrophenyl hydrazine, which produce a reddish brown compound, Pyruvate hydrazone, at basic media. Absorption measured at 365 nm.

C-Assay system for succinate dehydrogenase (SDH) activity: SDH (EC1.3.99.1) activity was assayed using a method adopted by Al-Hasany(2004). Flavine adenine dinucleotide (FAD) reduced to $FADH_2$ by succenic acid (SA), giving bright yellow color. Formation of NADH is propertional to the succinate dehydrogenase activity, which is mesured spectroscopically as increase in absorbance at 375nm. Concentration of consumered FAD was estimated according to Lambert-Bear rule: C=A/Ɛ. Unit of enzyme activity expressed as nM FAD/min/mg protein.

D- Assay system for fumarate reductase (FR) avtivity: FR, (EC 1.3.99.1) activity was assayed according to modified method described by Denicola-Seoane *et al*.(1992), addopted by Chen, *et al*. (2001) and Al-Hammoshi (2006). The method depends on absorbancy decrease of the reduced form of NADH at 340nm, in the presence of fumarate (the substrate), and crude enzyme in the worm extract of the worms. The reaction was monitored spectroscopically at 37 ºC. The decrease in absorbance of NADH was registered from zero-time for 10 minutes. Unit of enzyme activity was expresed as the ammount of enzyme required for the oxidation of 1µM of NADH/minute of reaction, depending on NADH extention coeffecient which is $6.2\times10^3\ M^{-3}cm^{-1}$.

VI-Statistical analysis:Student test was used for the analysis of data to compare differences between any two treatments (Steel and Torri, 1980). Statistical package for sociaty (spss) program, version 12 was sought to analyse the data by computer. All results were significant with $P\leq0.05$.

Results and Discussion

Biochemical estimation and comparisom for the energy occumulated macromolecules in intestinal cestodes i.e. *O. bofonis* and *C. columbae* were expresed in table. 1. Generally, concentration of carbohydrate were higher than that of lipides and protiens in the two examined helminthes. On the other hands, there were significant differences at $p\leq0.05$ between the concentration of carbohydrates, lipides and protienes of the tow cestodes, which were, 153 µg/ml , 105 mg/100ml and 113 µg/ml, respectively in *O. bofonis*, and 240 µg/ml , 196 mg/100ml and 181 µg/ml , respectively in *C.columbae*, depending on the wet weight. Eventually, concentrations of the three macromolecules were higher in the pigeon cestode.

Table(1): Concentration of total carbohydrtes, lipids and proteines in *Ophiotaenia bofonis* and *Cotugnia columbae* extract*:*

Cestode	**Carbohydrates conc.μg/ml**	**Lipids conc.mg/100ml**	**Protiens conc.μg/ml**
Ophiotaenia bofonis	153**±11.31	105**±9.21	113**±8.48
Cotugnia columbae	240±13.56	196±6.83	181±9.17

*Each value represent mean of three replicates±SE.

**Reffered to presence of significant differences between the values at P≤0.05, according to t-test, two sample analysis.

The present resultes some what consisted with that of Al-Naftachi (2006), who compared biochemicaly between intestinal cestodes of four vertebrate hosts, *Bothreiocephalus sp* (in *Barbus luteus* fish), *Ophiotaenia europaea* (in snake), *Raillietina echinobothrida* (in gull bird), *and Moniezia expansa* (in sheep*)* .She revieled that carbohydrates had the hiest concentration than protienes and lipides in the four examened helminthes. Wereas lipids had the less percentage of the cestode wet wight. She gathered this result with the role of carbohydrates in these unaerobic intestinal parasites as main energy reservior. The same as for Biswal, *et.al*. (2012), who stated that carbohydrates has the highest percentage of dray weight (25.2%) in *Cotugnia cuneata* (the intestinal cestode of domestic pigeon), followed by protien (21.3%), and lipid (9.9%), he concluded that glycogen was the most domenant form of carbohydrates. Furthermore, Sonune (2012) pointed out that percentage of lipids is higher in the gastrontestinal cestode of *Ovis bahral* sheep, than carbohydrates and protienes. He reffered to the importance of lipides in these helminth as energy source and in ova formation. Waghmare and Chavan (2010) stated that *Cotugnia digonopora* cestode depends on anaerobic carbohydrate metabolism to obtain the energy required ,a regular supply of glycogen is necessary .Hence large quantities of polysaccharide are stored which can be oxidized to yield ATP.

On the other hands, The present resultes agree with that of Al-Naftachi (2006) who elucidated that concentration of carbohydrate in gull bird intestinal cestode (*Raillietina echinobothrida*) was higher than that of snake cestode (*Ophiotaenia europaea*). But not agree with the same researcher of that proteines and lipids concentration in snake cestode were higher than that of gull bird cestode.

HPLC analysis of lipids revealed that *O. bofonis* adult worm contain six types of fatty acides depending on retention time of the authentic fatty acid esters (figure.1 and Table.2&3). Three were saturated :myristic C14:0(0.89%); palmatic C16:0 (2.29%); and stearic acid C18:0 (2.72%). The others were non-saturated acids : oliec C18:1(7.91%) ; linoliec C18:2 (73.12%) and lenolenic C18:3(13.07%). The unsaturated Linoleic and linolenic acids were the most domenant types.

Fatty acid analysis of *C.columbae* adult worm also, expose six types of fatty

acids (Figure.2 and table 2&3). Four fatty acides were saturated :capric C10:0 (0.19%); myristic C14:0 (2.38); palmatic C16:0 (45.86%) and stearic acid C16:0(51.42%), in addition to two unsaturated fatty acids: oliec C18:1(0.06%) and linoliec acid C18:2(0.09%). The saturated stearic and palmatic acids were the most domenant types.

Table(2): Retention times (minutes) of authentic fatty acids esters

	Saturated fatty acids				Non-Saturated fatty acids		
Fatty acid	Ca C10:0	My C14:0	Pa C16:0	St C18:0	Ol C18:1	Li C18:2	Ln C18:3
Retention time(minute)	6.106	6.649	7.712	8.401	10.151	10.925	12.210

Ca: Capric acid **My**: Myristic acid **Pa**: Palmatic acid **St**: Stearic acid

Ol: Oleic acid **Li**: Linoleic acid **Ln**: Linolenic acid.

Table(3): Types and percentage% of fatty acids found in *Ophiotaenia bofonis* and *Cotugnia columbae* extract

Cestodes	Fatty acids%						
	Ca	My	Pa	St	Ol	Li	Ln
Ophiotaenia bofonis	0.0	0.89	2.29	2.72	7.91	73.12	13.07
Cotugnia columbae	0.19	2.38	45.86	51.42	0.06	0.09	0.0

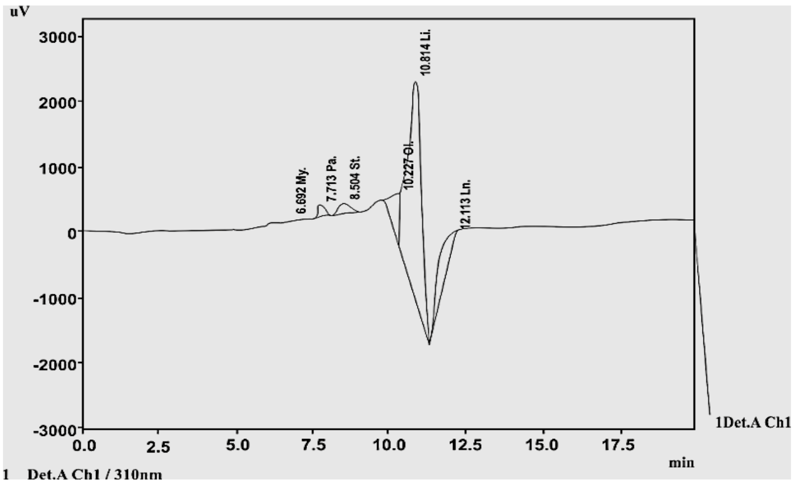
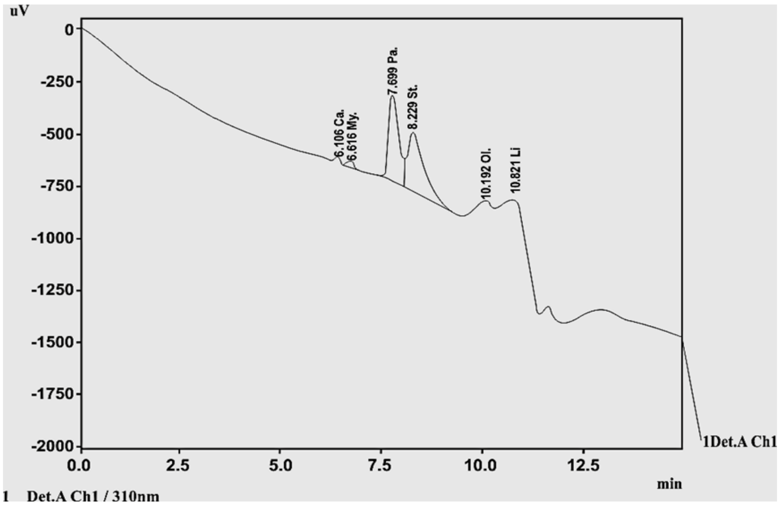


Figure (1): Chromatogram of fatty acid esters, which were separated from the extract of frog cestod, *Ophiotaenia bofonis.*



Figure(2): Chromatogram of fatty acid esters, which were separated from the extract of pigeon intestinal cestod, *Cotugnia columbae*.

Thus, C16 and C18 were the most common types of fatty acides in the two studied cestodes , they may represent the main absorbed fatty acids through body wall, those converted to the other type of fatty acids within the parasite body. This is come with some consistance with the results of Aisien and Ogiji (1989), who analyze fatty acides of th lizard intestinal cestode, *Oochoristica agamea.*They adverted that total lipids of the helminth fresh weight were 8.4%, and that 16 and 18-carbon fatty acids were the most abundant fatty acids. The same as for Mondal *et al*. (2009) who analyses fatty acids composition of *Raillietina echinobothrida*, the intestinal cestode of fowl (bird), they referred to presence of 18 fatty acids, and that chaif components fatty acids were palmatic (C16) and stearic acids (C18). It is reported that cestodes are able to absorb both short and long –chain fatty acides directly from host intestine, through a mixture of diffution and mediated transport (Smyth and McManus, 2007). Smyth (1994) stated that fatty acid synthesis in cestodes is restricted to the chain lengthining of host derived fatty acides by sequential addition of acetyl-CoA. Jacobsen and Fairbarin (1967) demonstrated that *Hymenolepis diminuta* can convert palmate and stearate into unsaturated fatty acids as long as 26 carbon. Elongation of C16, C18, C18:1, C18:2, C18:3 to C20 and C22 fatty acids was demonstrated also in *Spirometra mansonoides* cestode (Myer, *et al*. 1966).

Glucose, fatty acids and amino acids are the three substrates which organisms can use to maintain metabolic homeostasis. They are aquired for the generation of energy, but also as a building blocks for the biosynthesis of the macromolecules.

In the present work, the variant concentration of carbohydrates, proteines, lipides and fatty acides in the extract of the two cestod *O.bofonis* and *C. columbae* may related to the variation between the frog and pigeon considering nutritional habits and the physiological differences between the

two host; domestic pigeon, a bird that is endothermal (warm-blooded) animal who need excessive metabolic rates allover the seasons. (White, etal 2007); and house frog, an amphebian animal that is poikilothermal (cold-blooded) animal, who need variant basic metabolic rates between hibernation season and activity season(Moore, 1964). Smyth and McManus (2007) endicated that glycogen (the most important energy reserve in cestodes) fluctuates over a wide range, due to factor's such as season's ,physiological state of the host, the time of autopsy ,strain of the host, rate of the infection and stage in life cycle.

In parasitic helminths, the relationship between diet and fatty acides composition is particularly marked in cestodes (Barrett, 1983). Smyth and McManus (2007) reffered also to the high variation in lipid contents of the cestodes. And that the ability to synthsize fatty acids *de novo* in cestodes has been very much restricted and thus they have to depend largely on the fatty acids of the host to fullfill their requirments. George, *et.al*. (1969) were analyse fatty acids composition of five intestinal cestodes, obtained from 5 species of shark. They find out that there was complete qualitative correspondance between host and parasite acids, including the distinctive C_{20}-C_{22} polyunsaturated acides. As for C_{16} and C_{18} acides, they observed significant qualitative differences. George, *et.al.* (1971) were examined lipid content for three species of cestodes in leopard shark and brown smooth hound shark. They find out that there were obvious relationship between the present fatty acids and the marine food chain.

As for energy metabolism, parasitic stages of helminths do not use lipids normally as energy source reserve. When glucose supply become limitted, body organs can use fatty acides directly to generate energy (Houten and Wanders, 2010). Fraga *et al* (2012) found that treating mice artificially infected with *Taenia crassiceps* cysticerci, with low dose of albendazole, resulted in block of glucose uptake by the larvae, the event that induced fatty acids oxidation as energy alternative source ie. lead to metabolic alterations in the larvae. that reffered to a great host-parasite interaction. Additionally,the adult cestodes take large ammount of lipids to obtain suffecient ammount of fatty acids and fat-soluble vitamines and the access lipid is stored and eventually excreated (Smyth ,1994) . Stored lipides are the source of energy indispensable for the formation of the cyst tissue in *Hymenolipis dimenuta* (Moczon, 2006). Lipids play an important role in long-term adaptation and completion of the life cycle during their endoparasitic stage (Sato, *et al*. ,2008).

Mitochondria is the main site for oxidation of plasma free fatty acids or lipoprotein-associated triglycerides (see Wanders, *et al*. 2010). Mitochondrial fatty acid β-oxidation (FAO) pathway play a pivotal role in the energy homeostasis, but it compete with glucose as a primary oxidative substrate. The mechanism behind this so-called glucose-fatty acid cycle or Randle cycle (Bartlet and Eaton, 2004). It is interesting to know that β-oxidation of one molecule of stearic acid (C:18) will give 120 ATP molecules, while oxidation of one glucose molecule (C:6) will give 32 ATP molecules. Thus, Fatty acids have a higher ATP yield/Carbon atom than carbohydrates. Besides, ATP yeald of saturated fatty acid is higher than that of unsaturated

fatty acids (*Houten*, and Wanders, 2010).

On the other hands, the cestodes utilize different degrees of protein for producing energy. Litruchers revealed that the parasites able to adopt themselves to the parasitic mode of life, only due to protein (Jadhav, 2008).

In the present work, enzymatic studies depending on colorometric methodes, generally pointed to the significant difference between activity of the four estimated enzymes (G6PDH, LDH, SDH and FR) at p≤0.05 in the two gastrointestinal cestodes (table.4). Unit of enzyme activity for G6PDH and LDH in cellular extract was higher in *O. bofonis* , 7.515 U/L and 65IU/L respectively, than that of *C. columbae* ,4.856 U/L and 37 IU/L respectively. The same as for the activities of SDH and FR, which were higher in the frog cestode , 130 nM FAD/min/mg protein and 198 nMNADPH/min/mg protein, respectively than that found in the pigeon cestod, 69nM FAD/min/mg protein and 133nM NADPH/min/mg protein respectively.

Table(4): activity of G6PD,LDH, SDH, FR in *Ophiotaenia bofonis* and *Cotugnia columbae* adults extracts

Cestodes	**Enzyme activity**			
	G6PDH U/L	**LDH IU/L**	**SDH nM FAD/min/mg protien**	**FR nM NADH/min/mg protien**
Ophiotaenia bofonis	7.514**±1.38	65**±3.8	130**±11.13	198**±6.15
Cotugnia columbae	4.856±0.62	37±5.5	69±5.14	133±7.4

*Each value represin ent mean of three replicates±SE.

**Reffered to presence of significant differences between the values at P≤0.05, according to t-test, two sample analysis.

It is appeared that the cytosolic enzyme, G6PDH activity were very low in the extract of the two studied cestodes (Table. 4). This result consisted with those recorded by Pampori and Srivasta (1987) , who found that G6PDH was present in very low concentration in *Cotugnia digonopora*, a fowl intestinal cestode. In the present work, the low concentration of G6PDH in the the two cestodes (Table. 4) may reffered to that this enzyme involved in rate limiting steps for controlling the extent of glycolisis or glycogen synthesis, since that G6PDH is the very first enzyme of pentose phosphate shunt (Tian, et al, 1998) , a metabolic pathway that supplies reducing energy to cells by maintaining the level of the co-enzyme NADPH. G6PDH reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate. Von Brand (1979) manifested the exestance of functional

pentose phosphate cycle in *Echinococcus granulosus, Ascaris lumbricoides, Hymenolepis dimenuta* and *Fasciola hepatica*.

Al-Naftachi revieled that G6PDH activity in *O. europaea* (snake cestode) were higher than that of *Railletina echinobothria* (avian cestode), she combined this result with the inherited variation. This is agree with the present study that G6PDH activity were higher in frog cestode than that of pigeon cestode.

LDH activity in the two studied helminthes were higher than that of G6PDH of the same helminthes (Table. 4), this result some what simmilar to those reported in other parasites (Von Brand, 1979 and Pampori and Srivasta ,1987). LDH brings about reduction of pyruvate and results in production of lactic acid and thereby supplying an ATP molecule. Waghmare and Chavan (2010) bind the relatively high activity of LDH in *C. dignopora* adult with the role of this enzyme in the energy supply and in the maintenance of the cytoplasmic redox state. Waghmare and Chavan (2010) mentioned the important role of pyruvate in the energy metabolism of cestode, pyruvate may converted to lactate by the action of LDH which is excreted out along with energy rich product which utilized by the host.

SDH and FR were found in significant concentrations in the tow studied worms (Table.4), indicated that these cestodes are aerobic fermenters, in which FR activity provide succinate , which in turen used by SDH in the opposite direction , also fumarate involving this enzyme system giving rise to succinate, could act as terminal electron accepter (Bryant and Behm, 1989). Several cestodes were proved to accumulate succinate like: *Hymenolepis diminuta, Moniezia expansa, Echinococcus granulosus* cyst, *Taenia taeniaformis* adult and larvae (Saz, 1970). Pampori, *et al.* (1984) stated that fumarate and succinate induced significant production of ATP in *Cotugnea dignopora* adult cestod, and that a net phosphorylation of approximately 2 mol of ADP was observed for each mol of CO_2 liberated from malate or succinate. Pampori and Srivasta (1987) endicated also that FR and SDH, the mitochondrial enzymes were found in a significant concentrations in *C. dignopora*. Waghmare and Chavan (2010) reffered to that presence of high level of SDH activity in *Cotugnia digonopora* suggesting the existence of CO2 fixation pathway or partial reverse of Kreb's cycle. In the present study, the higher activity of SDH and FR in *O. bofonis* than that in *C. columbae* reffered to that carbohydrate dissimilation in frog cestode and so ATP yeild is more active than that in pigeon cestode.

This variance in enzymes activity in the two helminthes may reflect the variance in metabolic rates of their host, specislly during activity and proliferation seasons.

Conclusion

1-The occumulated sources of energy (carbohydrates, lipides, and protienes) semed to be more available in *Cotugnea columbia* (the pigeon cestode). Semeoltaneously, the activity of energy releaving enzymes (G6PDH, LDH, SDH and FR) were higher in *Ophiotaenia bofonis* (the frog cestod). These variance between the two cestodes may related to the variance of enveronmental conditions within there habitats.

2-HPLC analysis for fatty acids showed that the unsaturated fatty acides, stearic and palmatic, were the most common types in *Ophiotaenia bofonis* . Were as, the saturated linolic and linolenic fatty acids were domenant in *Cotugnea columbia*.

3- Availability of fumarate redactase, i.e, succenic acid in the two intestinal cestodes may point out the role of this component as end or intermediate product that can re-enter Kreb`s cycle.

Recommendations

1-There is a need for further investigation about lipids metabolism in *Ophiotaenia bofonis* and *Cotugnia columbae*, and wether fatty acides elongation take place by reversal β-oxidation, or by carboxilation of acetyl-CoA to malonyl-CoA, then subsequently added to pre-existing fatty acides.

2-Tracing pyruvate fathe may reflect path ways of energy production in these worms. For example detecting activity of pyruvate decarboxilase, and pyruvate dehydrogenase enzymes may aid in assesment of pyruvate fathe in these worms.

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