

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 $\mu\text{g/ml}$ and 196mg/100ml, respectevly in the pigeon cestode and 105, 153 $\mu\text{g/ml}$ 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 relatively 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 abundant 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 costly 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 ecosystem in which the host themselves live (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 environment around the parasite is the availability 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, propanoate 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 substancially different from that of their hosts. Apparently, they have adapted to exploit anaerobic niches and the surplus substrate that are commonly present in their environment. 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), others were taxonomic

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

Therefore, this research aimed to compare between the some aspects of energy metabolism of *Ophiotaenia bofonis* as an amphibian intestinal cestode and *Cotugnia 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 Ninivah Governorate, 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 brought and dissected in the research lab/Biology Department/College of Science/University of Mosul.

The collected cestodes were washed several times with NaCl buffered saline 0.85%, put in petridish, examined under Hamilton dissecting microscope. wet weight for each sample was fixed. Then kept in a deep freeze at -18°C for subsequent studies. Some helminthes were squized and fixed in hot formalin 10%, then dehydrated with gradient 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 weight/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 suspension. Ultracentrifugation at 15000g/30minutes was done using MSE superspeed ultracentrifuge. The supernatant fraction was chosen 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 ethylenediaminetetraacetic acid (EDTA). Worm's tissue were granded in a concentration of 10% (W/V), the homogenate then centrifuged at 1000g for 20 minute, the supernatant then recentrifuged at 15000g for 30 miute. The precepitate 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 disrupted by four successive barests 30 second (with 1 minute cooling intervals) using

ultrasonic disintegrator, all the above operations were carried out at 4°C.

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 measured at 488nm. Carbohydrate concentration were estimated depending on carbohydrate standard curve.

B-Estimation of total proteins:

Lowry, *et al.* colorimetric method (1951) was used to estimate protein concentration in the worms extract. Foline reagent reacts with protein to produce blue complex in alkaline medium. Absorbance was measured at 750nm. Concentration of protein was estimated using protein standard curve.

C-Estimation of total lipids: Chabrol and Chardonnet colorimetric method (1937) was used to evaluate whole lipids content (cited by Tietz, 1980). 20 µL of worms extract was heated with concentrated sulphuric 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:

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

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 were dissolved in 10ml of 7.5N NaOH (which prepared in 60% methanol). The mixture then heated to 105°C for 90minutes, after cooling, 12ml of distilled water added, the mixture then acidified using 20% sulphuric acid to adjusted pH to 2. Fatty acid mixture withdrawn with

separating funnel after mixing with diethyl ether. Rotary vacuum evaporator was used to extract fatty acid mixture.

B-HPLC analysis for fatty acids:

High performance liquid chromatographic technique (HPLC)-reversible 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 acid esters and authentic fatty acids were analysed using the following conditions:

C18 column (4.6×250)mm, mobile phase: (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 measured using Tietz spectrophotometric modified method (1995). Formation rate of NADPH is proportional to the G-6-P activity, which is measured 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 between the 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 FADH₂ by succinic acid (SA), giving bright yellow color. Formation of NADH is proportional to the succinate dehydrogenase activity, which is measured spectroscopically as increase in absorbance at 375nm. Concentration of consumed FAD was estimated according to Lambert-Bear rule: $C=A/\epsilon$. Unit of enzyme activity expressed as nM FAD/min/mg protein.

D- Assay system for fumarate reductase (FR) activity: FR, (EC 1.3.99.1) activity was assayed according to modified method described by Denicola-Seoane *et al.*(1992), adopted by Chen, *et al.* (2001) and Al-Hammoshi (2006). The method depends on absorbance 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 °C. The decrease in absorbance of NADH was

registered from zero-time for 10 minutes. Unit of enzyme activity was expressed as the amount of enzyme required for the oxidation of 1 μ M of NADH/minute of reaction, depending on NADH extinction coefficient which is $6.2 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

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 society (spss) program, version 12 was sought to analyse the data by computer. All results were significant with $P \leq 0.05$.

Results and Discussion

Biochemical estimation and comparison for the energy accumulated macromolecules in intestinal cestodes i.e. *O. bofonis* and *C. columbae* were expressed 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 $p \leq 0.05$ between the concentration of carbohydrates, lipides and protienes of the tow cestodes, which were, 153 $\mu\text{g/ml}$, 105 mg/100ml and 113 $\mu\text{g/ml}$, respectively in *O. bofonis*, and 240 $\mu\text{g/ml}$, 196 mg/100ml and 181 $\mu\text{g/ml}$, 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 carbohydrates, lipids and proteins in *Ophiotaenia bofonis* and *Cotugnia columbae* extract:

| Cestode | Carbohydrates conc.µg/ml | Lipids conc.mg/100ml | Protiens conc.µg/ml |
|----------------------------|--------------------------|----------------------|---------------------|
| <i>Ophiotaenia bofonis</i> | 153**±11.31 | 105**±9.21 | 113**±8.48 |
| <i>Cotugnia columbae</i> | 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 $P \leq 0.05$, according to t-test, two sample analysis.

The present results some what consisted with that of Al-Naftachi (2006), who compared biochemically 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 revealed that carbohydrates had the highest concentration than proteins and lipids in the four examined helminthes. Whereas lipids had the less percentage of the cestode wet weight. She gathered this result with the role of carbohydrates in these anaerobic intestinal parasites as main energy reservoir. The same as for Biswal, *et.al.* (2012), who stated that carbohydrates has the highest percentage of dry weight (25.2%) in *Cotugnia cuneata* (the intestinal cestode of domestic pigeon), followed by protein (21.3%), and lipid (9.9%), he concluded that glycogen was the most dominant form of carbohydrates. Furthermore, Sonune (2012) pointed out that percentage of lipids is higher in the gastrointestinal cestode of *Ovis bahral* sheep, than carbohydrates and proteins. He referred to the importance of lipids 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 results 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 proteins 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 acids depending on retention time of the authentic fatty acid esters (figure.1 and Table.2&3). Three were saturated :myristic C14:0(0.89%); palmitic C16:0 (2.29%); and stearic acid C18:0 (2.72%). The others were non-saturated acids : oleic C18:1(7.91%) ; linoleic C18:2 (73.12%) and linolenic C18:3(13.07%). The unsaturated Linoleic and linolenic acids were the most dominant types.

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

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acids (Figure.2 and table 2&3). Four fatty acids were saturated :capric C10:0 (0.19%); myristic C14:0 (2.38); palmitic C16:0 (45.86%) and stearic acid C16:0(51.42%), in addition to two

unsaturated fatty acids: oleic C18:1(0.06%) and linoleic acid C18:2(0.09%). The saturated stearic and palmitic acids were the most dominant types.

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

| Fatty acid | Saturated fatty acids | | | | Non-Saturated fatty acids | | |
|------------------------|-----------------------|-------------|-------------|-------------|---------------------------|-------------|-------------|
| | 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:** Palmitic 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% | | | | | | | | | |
|----------------------------|------|------|-------|-------|--------------|------|------|------|------|-------|-------|--|--|--|
| <i>Ophiotaenia bofonis</i> | | | | | Ca | My | Pa | St | Ol | Li | Ln | | | |
| | | | | | 0.0 | 0.89 | 2.29 | 2.72 | 7.91 | 73.12 | 13.07 | | | |
| <i>Cotugnia columbae</i> | 0.19 | 2.38 | 45.86 | 51.42 | 0.06 | 0.09 | 0.0 | | | | | | | |

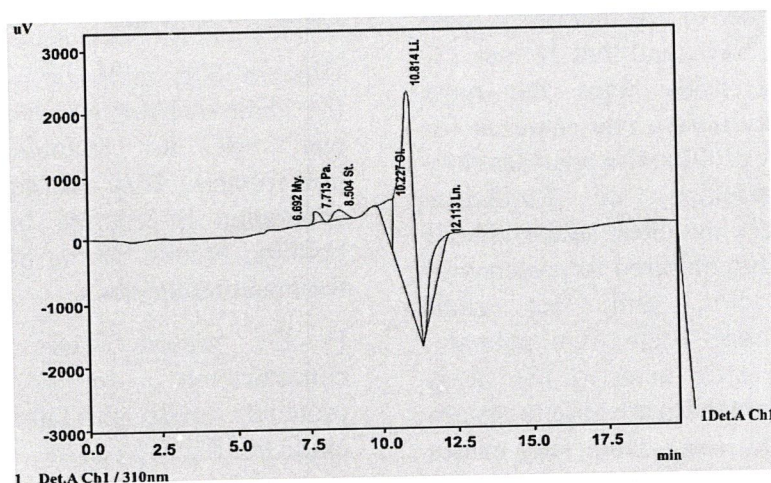
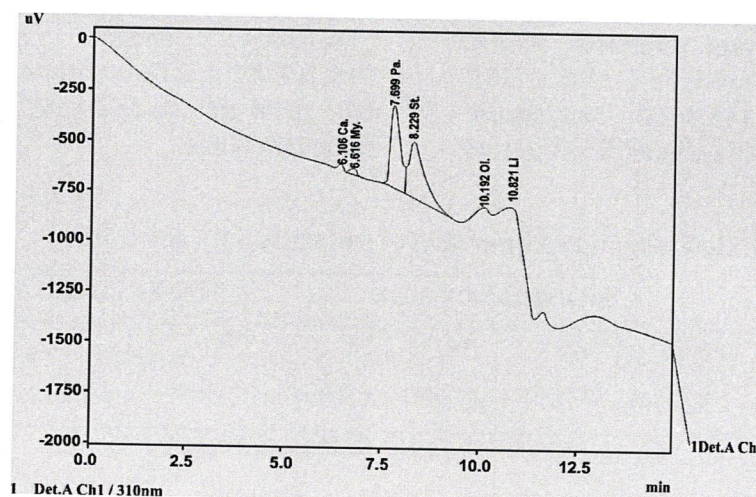


Figure (1): Chromatogram of fatty acid esters, which were separated from the extract of frog cestod, *Ophiotaenia bofonis*.



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 acids 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 consistence with the results of Aisien and Ogiji (1989), who analyze fatty acids of the 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 palmitic (C16) and stearic acids (C18). It is reported that cestodes are able to absorb both short and long-chain fatty acids directly from host intestine, through a mixture of diffusion and mediated transport (Smyth and McManus, 2007). Smyth (1994) stated that fatty acid

synthesis in cestodes is restricted to the chain lengthening of host derived fatty acids 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 mansonioides* 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 acquired 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, proteins, lipids and fatty acids in the extract of the two cestodes *O. bofonis* and *C. columbae* may be 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 all over the seasons. (White, et al 2007); and house frog, an amphibian 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) indicated that glycogen (the most important energy reserve in cestodes) fluctuates over a wide range, due to factors 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 acids composition is particularly marked in cestodes (Barrett, 1983). Smyth and McManus (2007) referred also to the high variation in lipid contents of the cestodes. And that the ability to synthesize 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 fulfill their requirements. 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 C₂₀-C₂₂ polyunsaturated acids. As for C₁₆ and C₁₈ acids, 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 limited, body organs can use fatty acids 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 referred to a great host-parasite interaction. Additionally, the adult cestodes take large amount of lipids to obtain sufficient amount of fatty acids and fat-soluble vitamins and the excess lipid is stored and eventually excreted (Smyth, 1994). Stored lipids 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 (Bartlett 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 yield 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 $p \leq 0.05$ 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 |
| <i>Ophiotaenia bofonis</i> | 7.514**±1.38 | 65**±3.8 | 130**±11.13 | 198**±6.15 |
| <i>Cotugnia columbae</i> | 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 $P \leq 0.05$, 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 glycolysis 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 diminuta* and *Fasciola hepatica*.

Al-Naftachi revealed 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 acceptor (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 CO₂ 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, specisly 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 acids, stearic and palmitic, 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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