FUDMA Journal of Sciences (FJS)
ISSN Online: 2616-1370
ISSN Print: 2645-2944
Vol. 2 No.3, September, 2018, pp 224 - 232

IMPACT OF *SCHISTOSOMA HAEMATOBIUM* INFECTION AND STARVATION ON SOME NEUTRAL AND POLAR LIPIDS CONTENT OF *BULINUS TRUNCATUS*

Eberemu, N. C.

Department of Biological Sciences, Federal University Dutsin Ma, Kastina State.

Corresponding Author: nkayy2@yahoo.com

ABSTRACT

Schistosoma haematobium infection has continued to be endemic in much of Africa and the Middle East, second only to malaria in importance as a parasitic disease.Thin layer chromatography (TLC) was used to study the effect of starvation and infection by miracidia of *Schistosoma haematobium* on the lipid content of *Bulinus truncatus*. *B. truncatus* snails were collected and kept in aerated plastic aquaria containing tap water. The progeny were transferred to other plastic aquaria where they were separated and designated as control/fed(c), infected (i), and starved(s). Urine samples of people infected with *S. haematobium* were collected and analysed for miracidia which were introduced into the aquaria labeled "infected". Only snails that shed matured brevifuvcate cercariae were used for the lipid analysis. The snails were homogenized and lipids were extracted in chloroform-methanol (2:1). The lipids extracts were analyzed using petroleum ether, diethyl-ether, and acetic acid (90:10:1). All the snails survived the 2weeks starvation. Visual chromatograms and Retention factor (Rf) values calculations showed that the major neutral lipids in infected, starved and control *B. truncatus* were fatty acids and phospholipids, wax and triglycerol were seen in stressed conditions. The value of total lipids in starved *B. truncatus* were approximately 2times lower than the control (3.66+0.00 < 6.79 ± 1.06g/dl) while value of starved snails were 5times lower than the control (1.94 ± 2.89< 6.79 ± 1.06g/dl). There were statistically significant differences in fatty acids, cholesterol and phospholipids in the samples, the mean differences > Fisher's Least Significant Different (F-LSD) value. In this study, triglycerol and wax were present in starved snails which implies that starvation were seem to distort the lipid composition of the snails

Keywords: *Schistosomahaematobium, Lipids, Bulinustruncatus, starvation, Thin Layer Chromatography*

INTRODUCTION

The snail intermediate hosts of schistosomes vary with the species of the worms. This also coincides with geographical distribution of the disease. The snail *Bulinus truncatus* is a species of air breathing freshwater snail; an aquatic pulmonate gastropod widely distributed in islands of the Mediterranean Sea, Portugal, Spain, Middle East as far as Iran and in Africa (Jørgensen *et al*., 2007 and Lange *et al*., 2013). *Bulinus truncatus* is the major snail host of *S. haematobium* in Nigeria (Ndifon andUkoli 1989; Emejulu *et al.,* 1994, Okafor and Ngang, 2004, Owojori *et al*., 2006). The natural habitat of *B. truncatus* has been greatly extended by development of vast agriculture production in Nigeria, especially in swampy areas. This has also propagated schistosomiasis. Schistosomiasis is prevalent in tropical and subtropical areas, especially in poor communities without access to safe drinking water and adequate sanitation (Okafor, 1990). It is estimated that at least 90% of those requiring treatment for schistosomiasis live in Africa (WHO, 2017). One of the means of fighting this disease is the eradication of snail vector (Okafor, 1990, Hamed, 2010).

The term lipids described or include the broad range natural material, which are soluble in organic solvent but are generally insoluble in water. Lipids are fats and oil. The two main groups of lipids are the natural lipids (triglycerol, fatty acid, alcohol, hydrocarbon and wax), complex or polar lipids (phospholipids, and glycolipids). The natural lipids tend to be part of energy store. Fatty acid is important source of energy for many organisms. Lipids exert important biological functions as energy storage compounds, structural components of the cell membranes and as signaling molecules (Zhukova, 2014).The unique fatty acid composition and chemical diversity of metabolites of some gastropods is determined by food supply and essential biosynthetic activities (Avila 1995, Zhukova, 2014).

Lipids also play various other important roles in the life of schistosomes (Skelly *et al*., 2014). Apart from being constituents of membranes, they also play roles in host recognition, cell signaling and development (Walker, 2011; Fusco *et al.,* 1988); immune response modulation and evasion (Salafsky and Fusco, 1987; Giera *et al.,* 2017), communication (Fried and Haseeb, 1991) and development (Shaw *et al.,* 1977). Some studies performed on the lipid metabolism of *Schistosomamansoni* have shown that the parasite is unable to synthesize fatty acids and sterols (Brouwers *et al.,* 1997), but is capable of forming complex lipids like phospholipids and triglycerol from precursors obtained from the host (Young and Podesta, 1982). The purpose of this work therefore; is to determine the effect of starvation and infection with *S. haematobium* cercariae on the lipid profile of *B. truncates* and also the distribution of the lipid

in starved and infected snails to help address the control of schistosoma intermediate host.

MATERIALS AND METHODS

Collection of snails and their Maintenance

B. truncatus (snails) were collected from Nkalagu (Niger cem) in Ebonyi State, Nigeria from marshy pools and "mkpa pits". They were collected during raining seasonon the month of July 2005. They were kept in 12 aerated plastic aquaria containing 1000ml of tap water (Pellegrino and Goncalves, 1965). Each plastic aquarium contained 10 snails. The water was changed once every week and the snails were fed with fresh lettuce. The snails were allowed to lay eggs, the eggs matured and hatched, the progeny were transferred to another plastic aquarium and were maintained as the adults.

A population of these progeny were designated as the control (c) and these were fed as mentioned above, their water changed once every week. Second groups infected (i) were fed and was infected with ***S***. *haematobium* miracidia. The third group starved (S) were kept without food for 2 weeks prior to test. Their water was changed every other day to remove dead snails; algae, mucus and excrement to make sure the snails do not feed on them.

Collection of Urine Samples

Urine samples of infected patients were collected using sterile sample specimen bottlefrom the same Nkalagu area. Paraense and Corred (1963), Kagen and Geiger (1965) have clearly shown that a snail serving as an intermediate host for a Schistosoma in one geographical area may be poorly susceptible or even refractory to infection with the same parasite from a different area. The urine samples collected were kept in a dark box to avoid hatching miracidia prior to the test. Samples, which contained blood, were lyzed by adding 2drops of saponin.

The urine samples were stirred thoroughly and 10ml were transferred into tubes, centrifuged at 1500-2000rpm for 5minutes to sediment the eggs. Pasteur pipette was used to remove the supernatant leaving the eggs. The eggs were hatched by diluting the sediment in warm tap water about 30^{o}C in a urine flask under a desk lamp. After 20min the water was decanted into a watch glass and miracidia were isolated by pipetting using a dissecting microscope (Trede &Becker, 1982). The figure 2 below shows the egg of *S. haematobium* found in the urine samples analysed.

Infection of Snails

Infections were carried out by exposing the *B. truncatus* to water containing miracidia. It was done by exposing the snails individually to about 10 miracidia each in small glass container with the minimum volume of water required for snail movement and at average temperature of 27^{o}C (Cram *et al.*, 1947). Successful infections are dependent upon the number of miracidia per snail and the temperature of the water (Dewitt, 1955). Only those snails that shed matured bifid cercariae were used for lipid analysis.

Extraction of Oil from the Samples

Oil from the tissues of the sample was extracted using Folch *et al.* (1957) method. 25 tissuecomplexes of *B. truncatus* with their shells were weighed. Each group was macerated using mortar and pestle until it was homogenized. The homogenized samples were extracted at room temperature with mixture of chloroform-methanol 2:1, v/v (150ml of chloroform to 75ml of methanol). The mixtures were blended for 1hr and 100ml of distilled water was added to it. The mixture was again blended for 30seconds and the homogenate was filtered with Whatman no 1 filter paper on a vacuum pump (sergeant-Welch Director V.P model 8805). The filtrate was transferred into a separating funnel to allow for complete separation and clarification. The chloroform layer, which contained oil, were then collected in a beaker and kept in a fume cupboard at a temperature not exceeding 50^{o}C under an atmosphere of nitrogen wash fluid to allow for evaporation of chloroform leaving the oil in the beaker. The percentages to the yield of oil were determined using the formula.

$$\% \text{ Yield of oil} = \frac{\text{weight of oil}}{\text{weight of sample}} \text{x } 100 \quad (1)$$

Preparation of Standard TLC Plates

Clean glass plates were clamped into the spreading rack and wiped with acetone. The adsorbent applicator was placed on top of the plates at one end of the rack. 50g of silica gel were shaken vigorously with 100ml of distilled water in a stoppered flask to form slurry and were quickly poured into the reservoir of the applicator. The plate was drawn smoothly across the other edges of the rack and was carefully removed from the spreading rack and transferred to the drying trays where they were allowed to dry at room temperature for 1hour. They were activated for use in a hot oven at 100^{o}C for 1hour (Renee and Joan, 1992).

Running the Thin Layer Chromatography (TLC)

The solvent system used for the TLC was made up of 100ml of petroleum ether, diethylether, and glacial acetic acid in the ratio 90:10:1 by volume and was poured into the dry chromotank. The edge of the tank was greased with Vaseline and covered to make it airtight. The solvent system was allowed to equilibrate for one hour. The lipid extract were reconstituted with 0.1% chloroform. Lipid from each group (starved, control and infected snails samples) was spotted 2cm from the bottom of edge of the coated plate using a capillary pipette. It was allowed to dry and was developed in the solvent system until the solvent had migrated approximately 2cm from the top of the plate. The plate was brought out and the solvent was allowed to evaporate. The plates were then transferred to the iodine tank to develop the spots. The relative distance of the spots was measured using a ruler as well as the distance moved by the solvent front. The characteristic mobility of each component described by its retention factor (R_f) value was determined from the relationship.

$$R_f = \frac{Dc}{DS} \quad (2)$$

Where DC = Distance moved by spot

DS= Distance moved by solvent front

R_f = retention factor

The R_f value of the various lipids component was then compared with the R_f value of standard lipid classes to identify the lipid components in the samples (Vroman and Baker, 1965, Smith *et al.*, 1966)

Determination of phospholipids content of the samples

The lipid fractions of phospholipids were extracted from each of the samples according to Zilversmith and Davis (1965). 0.5ml of each sample were pipetted into a test tube in duplicates and allowed to evaporate in a water bath. 1ml of perchloric acid was added into each of the test tubes. Blanks were prepared by adding 0.8ml of perchloric acid into a test tube. The samples were mineralized by heating. To analyze for phosphorous in the samples, 5ml of distilled water were added to each of the test tubes, the blanks and the standard solution. To each test tube, 1ml of 40g/L solution of ammonium molybdate was added followed by 1ml of aminonaphtile sulphuric acid reagent was added. Distilled water was added to bring the volume to 10ml and the solution thoroughly mixed. About 20min after, the absorbance (A) of the samples was determined. The samples were measured versus the blanks at 660nm wavelength.

The phospholipids is

$$P = \frac{A \text{ unknown}}{A \text{ standard}} \times 100 \text{ (mg/L)}$$

A = absorbance

The amount of phospholipids in mg/L is about 25x P

Determination of Triglycerol Content of the Samples

Triglycerides were determined using Carlson method (Carlson, 1963). 0.5ml of each sample was pipetted into a test tube in duplicates and 2.5ml of methanol was added to form slurry. To this slurry 5ml of chloroform was added followed by 7.5ml of normal saline. The tubes were closed, shaken vigorously and allowed to stand. The aqueous phase and the chloroform phase were clearly separated. The lower layer, which contained chloroform solution with the lipids, was withdrawn with a syringe fitted with needle. Spatula full of silica gel was added and allow to sediment. They were centrifuge for 2mins at 1000 x g. 0.5ml of the upper layer were collected and put into another sets of test tubes and 0.5ml of chloroform solution were pipetted into them including the blanks.

All the samples were heated in a water bath to evaporate. 0.1ml of ethanolic potassium hydroxide were added to each tube. The knob of the water bath were reduced to 6 i.e. 60^{o}C and left for 30min. After 30min, the samples were cooled to room temperature. 0.8ml of 0.7moles of sulphuric acid was added followed by 4ml of diethylether and shaken for 30min. The samples were separated; the upper layer was drawn off and discarded. From the remaining aqueous phase 0.3ml were pipetted into another test tube, 0.1ml of sodium periodate solution was added and shaken for 10min. 0.1ml of sodium arsenite solution was added and allowed to stand for 5min. There after 2.8ml of chromotropic acid reagent was added and heated in a water bath for 30min and then allowed to cool to room temperature. The absorbance of the various solutions was measured at 570nm against the blank.

Determination of Total Lipids Content in the Samples

Test tubes were labeled in duplicate according to each sample including the blank. 0.1ml of the samples was pipetted into test tubes and 2ml of conc. sulphuric acid was added to each sample. The tubes were swirled carefully, closed with a glass ball and were placed in a bath of boiling water for 10min. After cooling in cold water, 0.1ml of each sample was transferred into another test tube, 2.5ml of phosphoric acid vanillin reagent were added and the solution was mixed carefully. The intensity of the pink colour that developed reached its maximum after 30min and began to fade after about 50minutes. The absorbance of the samples were measured at 546nm against the blank (Zöllner and Kirch, 1962)

Determination of Fatty Acids Contents of the Samples

0.5ml of each sample was pipetted into a centrifuge tube with 5ml of chloroform and 2.5ml of copper reagent. In another tube, which served as the blank, 5ml of chloroform was added followed by 0.5ml of water and 2.5ml of copper reagent solution. The tubes were shaken vigorously for 2 to 3min and centrifuged for few minutes at 800 x g. After a few minutes of centrifuge, the aqueous and chloroform layer were separated. The aqueous phase was withdrawn with a syringe and discarded. The chloroform layer was left free of particles and its surface clean. 3ml of chloroform solution was pipetted into the test tube followed by 0.5ml of diethyldiothio carbamate reagent and mixed.The absorbance was measured at 440nm against the blank (Duncombe 1964).

Determination of Cholesterol Contents of the Samples

Test tubes were labeled in duplicate including the blanks according to Alexander and Griffiths (1992) protocol. 0.1ml of each sample was pipetted into a test tube with 3ml of glacial acetic acid. To the tubes, which served as blank, 0.1ml of distilled water and 5ml of glacial acetic acid were placed. The contents of the test tubes were mixed by gentle swirling and allowed to stand at room temperature for 30min. After cooling, 2ml of reagent solution was added to each tubes and mixed again. A light brown colour first appeared which changed to purple within a minute in all the tubes. After standing at room temperature for one hour, the absorbance of each was measured at 560nm against blank. The amount of total cholesterol was determined on an analytical curve, which was obtained by plotting the absorbance of the standard solution against their concentration.

Data Analysis

The result was analysed using analysis of variance (ANOVA), experimental error degrees of freedom using F-test for Completely Randomized Design (CRD). Fisher's Least Significant Different (F-LSD) or protected LSD and Duncan's new multiple Range test (DNMRT was used for the analysis

RESULTS

The figure one and two below represent the specie of the schistisoma intermediate host (*Bulinus truncatus*) used in this study and egg harvested after infection in this present work

Fig. 1 *Bulinus truncatus*

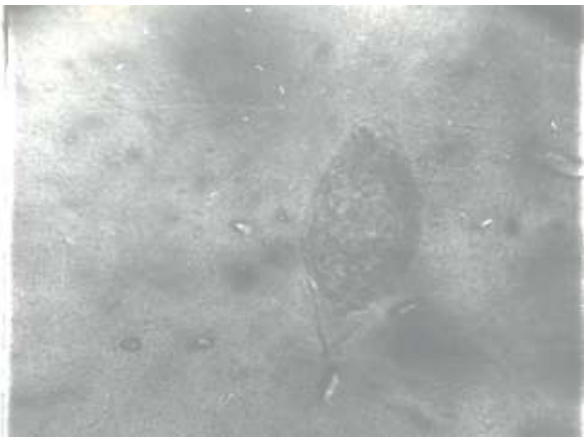

Fig 2: *Egg of Schistosoma haematobium*

Results of the Starvation and Infectivity

Outcome of the experiments showed that almost all the snails survived the 2 weeks of starvation. Among the 100 *B. truncatus* exposed to *S. haematobium* miracidia, 62 of the snails survived the 6 weeks post infections. These survivors were observed to be shading fully developed schistosomes-type bifid cercariae.

Total lipid of infected and starved *B. truncates* snails was compared with that of control snails as shown in Table 1.

There was a slight decline in the weight of the starved snails compared with the control and an increase in weight of the infected snails. They was a decline in total lipids of starved snail, which was about 5 times lower than that of the controls. The total lipid levels of the infected snails were two times lower than those of the control snails which is significant.

Table 1: Weight and Total Lipids Values of infected, starved and control/fed groups of *Bulinustruncatus*

Treatments	Weight of snails with shell (g)	No. of snails	Total lipids of snail (g/L)
Starved(S)	3.5	25	1.9363 ±2.887
Infected (I)	4.2	25	3.6570±0.000
Control (C)	4.0	25	6.7873±1.155

Table 1shows the R_f values of the treatments, the type of neutral and polar lipids present in *B. truncatus*. The lipid classes which showed that fatly acids and phospholipids were the main neutral lipid fractions present in *B. truncatus* and that wax and triglycerol were seen in stress condition (see Table 1).

Table 2: Distribution of lipids in experimental snails

Treatments	Lipids classes present	R_f values of samples
Infected (I)	Fatty acids (µg/dl)	0.52 ± 0.01
	Triglycerol (mg/dl)	0.60 ± 0.02
	Wax (g)	0.91 ± 0.02
	Phospholipids (mg/L)	1.00. ±0.00
Starved (s)	Fatty acids	0.50 ± 0.01
	Triglycerol	0.61 ± 0.01
	Wax	0.92 ± 0.01
	Phospholipids	1.00 ± 0.00
Control /fed (s)	Fatty acids	0.52 ± 0.00
	Phospholipids	1.00 ± 0.00

Calculating the R_f values of the samples separated on silical gel shows the type of lipids present in the snail. The triglycerol was slightly reduced (0.60 ± 0.02) in the infected snails compared to those of the starved snails which was (0.61 ± 0.01), even though the difference is not statistically significant. The phospholipids in all treatment have the same value. There is decline in fatty acids in starved snail.

Variations in mean concentration of phospholipids, triglycerol, total lipids, fatty acids and cholesterol in Bulinus truncatus under three experimental conditions Result of phospholipids concentration of the samples (Table 3) shows the phospholipids levels for all the sampled groups i.e. infected, starved and control/fed. Although the control and the infected snails showed no statistical significant differences, the infected snails showed a mean concentration of phospholipids that is slightly greater than the control snails. Starvation caused a decrease in the phospholipids mean concentration. There was a significant difference in phospholipid concentration between starved and the control snails. The result of triglycerol mean concentration was similar in all groups although, it is not significant.

The differences in total lipids mean concentration in the treatment/experimental groups was highly significant (P>0.00) (Table3), which were seen represented in different Although the relative mean concentration level of all the groups was significant, the control snail had the highest lipid concentration. The mean concentration of total lipid in infected snails was about half that of the control 6.79±1.06= 2 (3.66 ± 0.00). Starvation also decreased the total lipid concentration of the snail about five fold (1.94± 2.89) when compared with the control (6.79± 1.06). Total lipid content was least in starved snails. Total lipids level in infected snails was also less than that of the control, but greater than that of starved snails.

During starvation, there was a decrease in fatty acids. It also showed that they were mean variations between the groups, they were statistically difference between the relative mean concentration of the starved, infected and control/Fed of *B. truncatus*. Comparing the fatty acids in table 5, the control, starved and the infected, although there were reduction in the concentration level of fatty acids in starved snails, they was an increase in the infected snails which was statistically significant. All the mean concentrations of samples are in different subset, which also mean that it is highly significant ($P > 0.000$)

The overall mean concentration level of cholesterol varied among the groups. They were significant different within the groups comparing the control/fed (1507.01±21.64) with starved snail and infected snail, they were decrease in mean concentration of starved (800.54±107.33) and infected snails (839.12±165.64). Although the concentrations mean level of both starved snail and infected snail is not statistically significant still starvation lower the cholesterol level of the starved snail. Control/Fed has a highest level of cholesterol concentration so we can say in this work that infection and starvation lower the level of cholesterol in *B. truncatus.*

Table 3. Mean concentrations of phospholipids, triglycerol, total lipids, fatty acids and cholesterol in *Bulinus truncatus*among experimented groups

Lipids Classes	Mean Concentrations of different Sample groups					Anova test comparison within and between groups		
	Infected	Starved	Control/fed	P value	Remarks	1/C	1/S	S/C
Phospho-lipids (mg/L)	56.22±4.36 a	38.02±4.21 b	55.49±5.73 a	0.006	S	NS	S	S
Triglycerol (µm/g)	0.91± 2.30 a	0.59± 3.60 a	0.66± 0.55 a	0.47	NS	NS	NS	NS
Total lipids (g/L)	3.06±0.00 b	1.94±2.89 c	6.79±1.06 a	0.000	S	S	S	S
Fatty Acids (mM/g)	0.44±6.00 a	0.30±2.31 c	0.41±1.56 b	0.00	S	S	S	S
Cholesterol (mg/100ml)	800.54±107.33 a	839.12±165.64 a	507.01±21.64 b	0.00	S	S	S	S

S = significant, NS = non-significant,P-value = significant value, I/C = statistical significant between infected and control
I/S = statistical significant between infected and starved snail, S/C = statistical significant between starved and control/Fed snails.Mean or treatment not followed by the same letters are significant difference,Mean/underscored with the same the same letters are not signify.

DISCUSSION

There was no significant difference in the shell size and length of *B. truncatus* in this study in all the treatment group, probably because the shell are mainly calcium carbonate and not affected by lipid depletion. On the other hand there was a decrease in weight of the starved snail, which was also reported in the study by Mohamed *et al.* (1986). It was also observed that at the end of the experiment, the shell of the starved snails became delicate. According to Melinda *et al.* (1987), during starvation the mean total lipid values for *B. glabrata* declined to about 12%, but there is considerable variation in total lipids in individual snails. Although lipids were utilized by the schistosomes in *Bulinus africanus* during starvation, carbohydrates were preferentially oxidized (Heeg, 1977) and these may be the case with *B. glabrata* (Thompson, 1987). In the present study it was noted that there was a significant in total lipid level of *B. truncatus*, which was 5 times lower than the control. The lower level of lipids in tissues of starved *B. truncatus* suggests that lipids are utilized as a storage reserve, similar condition was reported by Streit (1978) in pulmonate *Arcylus fluviatilis* and Thompson (1987) in *B. glabrata*. Infection of *B. truncates* with *S. haematobium* in this study reduced the level of total lipids in starved snails, which is approximately 2 times the control. The observed increased in the triglycerol level fraction in infected snail is consistent with the finding of Cheng and Snyder (1962), James and Bowers (1967) and Porter (1970) all of whom demonstrated histologically an increased deposition of triglycerol in trematode infected molluscan tissue.

The observed TLC result y showed that the major lipids present in *B. truncatus* are fatty acids and phospholipids and that under conditions of stress further lipid classes were seen. Triglycerol were not seen in control, probably because it still intact in its stored stage, there is no need to use them since there are regular supplies of food. The presence of a parasite derived lipids which subsequently hydrolyzes neutral lipids in trematode infected snails was reported by Cheng (1965). The presence of triglycerol and wax in infected and starved snails in the present study may be as a result of hydrolysis of neutral lipids by the parasite during infection and the utilization of stored lipids for energy during starvation. Freid *et al*. (1993) found that the major neutral lipid fractions of the brood sac of *L. variae* were triglycerol, free sterols and sterol esters and these fractions were qualitatively similar to the lipids found in the snail host *S. ovalis*. Finding from Fried *et al.* (1995) confirmed the presence of triglycerols and free sterols as major neutral lipids in the brood sac and host snail tissue. The TLC study by Melinda *et al*. (1987) indicated that triglycerol and free sterols are the main lipids of *B. glabrata* along with lesser amounts of free fatty acids and sterol esters. The discrepancy in lipid classes may be due to the solvent system, no solvent system will completely separate all naturally occurring lipids in a single development. It is difficult to determine if the triglycerol seen in host snails was obtained from the parasite through membrane formation during rapid multiplication stage of the parasitic life cycle. Whether the host is directly or indirectly induced to synthesize more neutral lipids in response to the nutritional demand of the parasite is unknown. Moreover, the parasites could have interfered with the host biosynthetic-pathway, thereby causing an inhibition of host lipid metabolism and a reduced total lipid composition during infection. According to Thompson and Mejia-scale (1993), the role of lipids as a storage reserve in gastropods is not clear, and the role of lipids in metabolisms of snails and the significance of lipid to developing larvae require further investigation. Schistosoma parasites appear to distort the neutral lipids composition of the snail host especially during infection.

Other studies by Thompson (1987) and Fried *et al. (*1995) also show initial increase of neutral fat in the host cell when parasites are present, although neutral fats are rapidly degraded to fatty acids, which however decline as infection progressed. Starvation would seem to distort the lipid composition of the snails. In this study triglycerol and wax were present in starved snails.

CONCLUSION

Adult *B. truncatus* shows good survival rate during starvation, there was no mortality during this present work; although the shell were delicate which probable might have more effect in the snail activities, if starvation continue more than the time used in this present. Therefore activities directed towards eradication should be carried out primary during the period of food scarcity (dry season).This study also shows that the total lipids content of *B. trancatus* drops when infected with *S. haematobium.*

REFERENCES

Alexander, R. R. and Griffiths, J.M. (1992). *Basic Biochemical Method*. Walter de Gruilter and co. 166-167pp. Mohamed, F. F. Bayomy, Rene van Elk and Joos Joosse. (1986). The effect of starvation and refeeding on egg laying synthetic activity of the albumen gland in bulinus tativeruncates, a snail vector of urinary schistosomiasis. *Comparative Biochemistry and Physiology*. *87A*(3) 607-612

Avila, C. (1995**).** Natural products of opisthobranch molluscs: A Biological Review. *Oceanography and Marine Biology*, **33**: 487–559

Brouwers, J.F.H.M., Smeenk, I.M.B., Van Golde, L.M.G. and Tielens, A.G.M. (1997). The Incorporation modification and turnover of fatty acids in adult *schistosoma mansoni. Molecular and Biochemistry Parasitology, 88*:175-185.

Carlson, L.A. (1963). Procedure: colometrc determination of triglyceride in serum. *Journal of Atherosclerosis Resources, 3*:334.

Cheng, T. C. (1965) histochemical observations on changes in the lipids composition of the American oyster: *crassostreavirginica* (gmelina) parasitized by the trmatode *Bucephalussp*. *Journal of Invertebrate Pathology 7,* 398-407

Cheng, T.C. and Snyder, R.W. (1962). Studies on host parasite relationship between larval trematodes and their hosts III. Certain aspects of lipids metabolism in *Helisoma trivolvis* infected with the larvae of *Glypthelmins pennsylvaniensis* and related phenomena. *Transactions of Royal Society of Tropical Medicine and Hygiene, 81*: 327-331.

Cram, E. B., Files, V. S. and Jowes, M. F. (1947). Experimental molluscan infection with Schistosoma mansoni and Schistosoma haematobium. *National Institute of Health Bullation. 189,* 106-108

DeWitt, W.B. (1955). Influence of temperature on penetration of snail hosts by *S. mansoni* miracidia. *Experimental Parasitology, 4*: 271-276.

Duncombe, W. G. (1964) *Colorimetric determination of non-esterified Fatty acid in plasma*. *Clin. Chem. Acta. 9, 122*

Emejulu, A. C., Alabaronye, F.F., Ezenwaji, N.M. and Okafor,F.C. (1994). Investigation into the prevalence of urinary schistosoma in Agulu Lake area of Anambra state, Nigeria. *Journal of Helminthology*, *68(2*): 119-123.

Folch, J., Lees, M. and Sloane Stanley, G.H. (1957). A sample method for isolation and purification of total lipids form animal tissues. *Journal of Biology and Chemistry. 226*:497-509.

Fried, B. and Haseeb, M.A. (1991). Role of lipids in *Schistosoma mansoni*. *Parasitology,*7: 59.

Fried, B., Beers, K. and Lewis, P.D. J.R. (1993). Lipids in the brood sac of *Leucochloridium variae* (Digenea, Leucochloridiidae) and its snail host *Succinea ovalis. International Journal of Parasitology*, *23*: 129-131.

Fried, B., Lewis, P.D. Jr. and Beers, K. (1995). Thin layer chromatography and histochemical analysis of neutral lipids in the intramolluscan stages of *Leucochloridium variae* (Digenea, Leucochloridiidae) and the snail host, *Succinea ovalis. Parasitology*, *81(1):* 112-114.

Fusco, A.C., Salasfsky, B., Ellenberger,B. and Li, L. H. (1988). *Schistosoma mansoni;* Correlation between mouse strain skin eiosinoid production and cercariae skin penetra*tion. Journal of Parasitology 74,253-261*

Giera, M., Maria M.M. K, Rico J.E.D., Evelyne S, Yvonne C.M.K. Cornelis H.H., Maria Y., and Bart E. (2017) The *Schistosoma mansoni* lipidome: Leads for immunomodulation. Analytica Chimica Actahttps://doi.org/10.1016/j.aca.2017.11.058

Hamed, M. A. (2010). Strategic Control of Schistosome Intermediate Host. *Asian Journal of Epidemiology*, **3**: 123-140

Heeg, J. (1977). Oxygen consumption and the use of metabolic reserves during starvation in *Bulinus* (physopsis) *Africans. Malacologia ,16*:547-560.

James, B. L. and Bowers, E. A. (1967). The effect of parasitism by the daughter sporocysts of cercaria *bucephalopsis haimeana* on the digestive tubules of the cockie, *Cardium eolule. Parasitology*, *57*: 67-77.

Jørgensen, A., Jørgensen, L. V. G., Kristensen, T. K., Madsen H. and Stothard, J. R. (2007). Molecular phylogenetic investigations of *Bulinus* (Gastropoda: Planorbidae) in Lake Malawi with comments on the topological incongruence between DNA loci. *Zoologica Scripta* 36: 577–585.

Kagan, I.G. and Geiger, S.J. (1965). The succeptibility of three stains of *Australorbis glabrata to Schistosoma mansoni* from Brazil and purtorico. *Parasitology*, *51*: 622-627.

Lange, C. N., Kristensen, and Madsen, H. (2013). Gastropod diversity, distribution and abundance in habitats with and without anthropogenic disturbances in Lake Victoria, Kenya. *African Journal of Aquatic Science,* 38(3): 295–304.

Melinda, D., Bernard, F. and Joseph, S. (1987). Lipids in fed and stared *Biomphalaria glabrata* (Gastropoda). *Comparative Biochemistry and Physiology*, *86A*: 663-665.

Mohamed, F. F. Bayomy, R V and Joos Joosse. (1986). The effect of starvation and refeeding on egg laying synthetic activity of the albumen gland in bulinus tativeruncates, a snail vector of urinary schistosomiasis. *Comparative Biochemistry and Physiology*. *87A* (3) 607-612

Ndifon, G. T. and Ukoli, F. M. A. (1989). Ecology of freshwater snails in South Western Nigeria. I: Distribution and habitat preferences. *Hydrobiologia **171***: 231-253.

Okafor , F.C. and Ngang, I. (2004). Freshwater snails of Niger cement, Nkalagu Eastern Nigeria: Observation on some demographic aspects of the schistosome-transmetting Bulinids. *Animal Research International*,1(2): 120-124.

Okafor, F.C. (1990). *Shistosomahaematobium* cercariae transmission pattern in fresh water system of Anambra State, Nigeria. *Angewandte parasitology*, ***31 (3):*** 159-166.

Owojori, O. J., Asaolu, S. O. and Ofoezie, I. E. (2006). Schistosomiasis: Water Contact Pattern and Snail Infection Rates in Opa Reservoir and Research Farm Ponds in Obafemi

Awolowo University, Ile-Ife, Nigeria. *International Journal of Zoological Research*, ***2(4):*** 323 – 333.

Paraense, W.L. and Corred, L.R. (1963). Variation susceptibility of populations of *Australorbis glabrata* to a strain of *S. mansoni. Kerta Institute of Medical Tropical S Paulo,5*: 15-22.

Parasites & Vectors, 2011**4**:203. https://doi.org/10.1186/1756-3305-4-203

Pellergino, J. and Goncalves, M.G.R. (1965). Simple method for collecting egg clutches of *Biomphalariaglabrata, Australorbis glabratas* and for rearing newly hatched snails. *Journal of Parasitology, 51*:1014.

Porter, C.A. (1970). The effect of parasitism by the trematode *Plagioporu virens* on the digestive gland of its snail host *Flumenilcola virens. Proc. Haelminthology soc wash, 37*: 39-44.

Renee, R. A. and Joan, M. G. (1992). *Basic Biochemistry Method.* Ithaca, New York

Salafsky, B. and Fusco, A.C. (1987). *S. mansoni*. A comparative of secreted versus non secreted eiosanoids as correlates of penetration and transformation. *Parasitology*,72: 394-404.

Shaw, J.R., Marshall, I., Erasmus, D.A., (1977) *S. mansoni*: in vitro stimulation of vitelline cell development of extracts of male worms. *Experimental Parasitology,42*: 14-20.

Smith, T. M., Brooks, T. J., and White, H. B. J (1966). Thin layer and gas-liquid chromatography analysis of lipid from cercaria of *Schistosoma mansoni. American Journal of Tropical Medicene and Hygiene. 15 307-313*Skelly PJ, Da'dara AA, Li XH, Castro-Borges W, Wilson RA.(2014) Schistosome Feeding and Regurgitation. PLoS Pathog.10(8):e1004246. doi: 10.1371/journal.ppat.1004246.

Streti, B. (1978). Changes in Protein lipid and carbohydrate content during starvation in the freshwater *Limet Ancylus fluviatilis*. *ComparativePhysiology, 123*: 149-154.

Thompson, S.N. (1987). Effect of *S. mansoni* on the gross lipid compostion of its *Biomphalaria glabrata. Comparative Biochemistry and Physiology, 87B*:357-360.

Thompson, S.N. and Mejia-Scales, V. (1993). Effect of restricted food intake on hemolymph glucose concentration and disgestive gland-gonad lipid level in the schistosoma vector *Biomphalaria glabrata* (say) (Gastropoda: planorbidae). *The Veliger,36*: 425-432.

Tread, G.and Becker, W (1982). Effect of starvation and infection with schistosoma mansoni on the release rate of free Amino acid (FAA) by Biomphalaria glabrata. *Comparative Biochemistry and Physiology. 73B* (2) 405-409

Vroman, N. E.,and Baker, G. L. (1965). Limits of detection of some lipids in thin layer chromatography. *Journal of Chromatography 18, 190-191*

Walker, J. A (2011) Insights into the functional biology of schistosomes

WHO (2017). Updates fact sheet on Schistosomiasis. Community medicine 4asses http://www.who.int/mediacentre/factsheets/fs115/en/

Young, B. W. and Podesta, R.B. (1982). Major phospholipids and phosphatidychloline synthesis in adult *Schistosoma mansoni. Parasitology, 5*, 169-172.

Zhukova, N. V. (2014).Lipids and Fatty Acids of Nudibranch Mollusks: Potential Sources of Bioactive Compounds. *Marine Drugs*,***12***, 4578-4592

Zilversmit, D. B. and David, A. (1950). Procedure; Estimation of total phospholipids in plasma. *Journal of Laboratory Clinical Medicine. 35, 155*

Zöllner, N. and Kirsch, K. (1962) Procedure; Absorptimetric determination of total lipids in serum. *Gas. Exp. Med.* 135, 545.