FUDMA Journal of Sciences (FJS)
ISSN: 2616-1370
Vol. 2 No. 1, April, 2018, pp 54-58

EFFECTS OF FOOD DEPRIVATION ON LIPID COMPOSITION IN *BULINUS TRUNCATUS*

Eberemu, N. C.[1]* and Nnodim, N. N. [2]

[1]* Department of Biological Sciences, Federal University Dutsin Ma, Kastina State.
[2] Department of Microbiology, Parasitology Unit. Renaissance University Ugbawka, Enugu State
Email: nkayy2@yahoo.com

ABSTRACT
The effect of food deprivation on lipid composition in *Bulinus truncatus* was studied. Snails were collected from Nkalagu (NIGERCEM), Ebonyi State, fed with lettuce and allowed to grow and reproduce. From the first generation, 50 snails were collected and introduced into another set of plastic aquaria, and kept without food for two weeks prior to the test and another 50 were fed with lettuce. Total lipid of the starved snails was 1.9363 ± 2.887 g/l while that of the fed snails was 6.7873 ± 1.155 g/l. Thin Layer Chromatography results revealed the presence of phospholipids and fatty acids in fed snails, while triglycerol and wax were only found in starved snails. The phospholipid concentrations of the fed and starved snails were 55.49 ± 5.72 g/l and 56.22 ± 456 g/l respectively. Fatty acid values in fed and starved snails were 0.41 ± 6.00g/l and 0.30±2.31 respectively.

Keywords: *Bulinus truncatus, Lipids, Schistosomiasis, Starvation, Thin Layer Chromatography,*

INTRODUCTION

The snail *Bulinus truncatus* (Audouin, 1827) 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 (Stothard *et al*., 2001, Remy and Arouna 2005; Jørgensen *et al*., 2007 and Lange *et al*., 2013). *Bulinus truncatus* is the major snail host of *S. haematobium* in Nigeria (Ndifon and Ukoli 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 propagated schistosomiasis. *Bulinus* species has been a plague to agriculture; it has also been implicated in the transmission of various diseases causative agents of man, domestic animals and various wild animals such as *Schistosoma heamatobium, Schistosoma intercalatum*, *Schistosoma bovis*, *Schistosoma curassoni*, *Schistosoma mattheei* and *Paramphistomum cervi* etc acquiring economic, medical, veterinary and social importance. Schistosomiasis is prevalent in tropical and subtropical areas, especially in poor communities without access to safe drinking water and adequate sanitation. 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).

Water-logged swamps are dried up during dry season, during this period, the snail hides in mud of void water canals. This greatly decreases the availability of food for the snails. 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). Therefore, the objective of this study was to determined lipid composition and the alterations that occur in *B. truncatus* snails during food deprivation.

MATERIALS AND METHODS

Collection of snails and their Maintenance

Specimens of snails identified as *Bulinus truncatus* (Brown *et al.,* 1971) were collected, cultured and maintained in 10 aerated plastic aquaria containing 1000 ml of tap water; with 10 snails per aquarium. They were maintained under the average temperature of 27°C and were fed with lettuce. The snails were allowed to lay eggs, mature and hatched; the progeny were transferred to another plastic aquarium and were maintained for three months. Adult *B. truncatus* were transferred into five plastic aquaria and were labeled starved and maintained without food for 14 days prior to the test. Water in the aquaria was changed every other day to remove dead snails; algae, mucus and excrement to make sure the snails do not feed on them.

Extraction of Oil from the Samples

To gravimetrically determine total lipids, 25 fed and 25 starved snails with a maximum shell diameter of 12 ± 4 mm were homogenized by maceration. The lipid of the homogenized samples was extracted at room temperature with mixture of 225 ml chloroform-methanol 2:1, v/v (Folch *et al*., 1957). 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°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.

$$\%\ \text{Yield of oil} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100$$

Thin Layer Chromatography

Thin layer chromatography (TLC) was run using 100 ml of petroleum ether/diethylether/glacial acetic acid in the ratio of 90:10:1 by volume, the lipid extract was reconstituted with 0.1% chloroform. The lipid Extract of 30 µg from fed and starved snail was spotted on separate origin, 2 cm from the bottom of a 20 × 20 cm pre-coated silica plate. The spots were developed in the same direction for a distance of 17 cm beyond the origin in a saturated glass rectangular chromo tank, in the ratio of 90:10:1 by volume, following the method of Skipski *et al*. (1965). The relative distance of the spots was measured as well as the distance moved by solvent front. The characteristic mobility of each component described by its retention factor (R_f) value was determined from the relationship.

$$R_f = \frac{DC}{DS}$$

Where DC = Distance moved by spot

DS = Distance moved by solvent front

R_f = retention factor

The R_f value of the various lipid components was then compared with the standard R_f value of lipid classes to identify the lipid components in the samples (Brown and Benjamine 1964, Vroman and Baker 1965 and Smith *et al.,* 1966)

Determination of the Lipid Profile of the Samples

Fatty acids from the lipid of both starved and fed snails was determined using Duncomb (1964). The absorbance was measured at 440 nm against the blanks. Phospholipid was also determined from fed and starve snails. The absorbance was measured at 660 nm against the blanks. The amount of phospholipids in mg/l is 25 × phosphorous determined from the analysis. Triglycerol was also determined and the absorbance of the various solutions was measured at 570 nm against the blanks (Smith *et al*., 1995).

DATA ANALYSES

The result was analyzed 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).

RESULTS

All snails survived the approximately 14 days of starvation. There was a decline in the total lipid of starved snails (1.9363 ± 2.887, which was about 5 times lower than that of the fed snails (6.7873±1.155) (Table 1). The body weight of starved snails was lower than that of the fed snails (Table 1).

Table 1 Weight and total lipid values in fed and starved *Bulinus truncatus*.

Treatments	Weight of Snails (g)	Number of Snail	Total Lipid of the snails(g/L)
Starved snails	3.5	25	1.9363 ± 2.887
Control snails	4.2	25	6.7873±1.155

Thin layer chromatography results of the fed and starved *B. truncatus* reveals the presence of lipid in the snails. Phospholipids and fatty acids are the only lipid present in the tissue of *B. truncatus*, while triglycerol and wax in addition to the above were seen in starved snails. The R_f values of the samples separated on the silica gel were calculated and presented in Table 2.

Table 2. Type of lipids and R_f values of lipid presence in starved and fed *Bulinus truncatus*.

Treatment	Lipid classes present (mg/dl)	R_f values of sample
Starved Snails	Fatty acids	0.50 ± 0.01
	Triglycerol	0.61 ± 0.01
	Wax	0.92 ± 0.01
	Phospholipids	1.00 ± 0.00
Control/Fed Snails	Fatty acids	0.52 ± 0.00
	Phospholipids	1.00 ± 0.00

The level of phospholipids and fatty acids showed in Table 3 demonstrates that starvation decreased the value of these lipids.

Table 3. Variations in the mean concentration of phospholipids and fatty acids in Bulinus truncatus

Lipid Classes	Starved Snails	Fed Snails	P value	Remarks
Phospholipids (mg/dl)	38.02±4.21	55.49±5.73	0.006	**S**
Fatty Acids (mg/dl)	0.30±2.31	0.41±1.56	0.00	**S**

DISCUSSION

Approximately, all the snails survived the two weeks starvation, there was a decline in weight of the snail and the shell of the starved snails became delicate when compared with the control snails at the end of the experiment. Vianey-Liaud and Lancastre (1984) reported no significant difference on snail's survival after four weeks of varied starvation time; although, half of the snail that survive the intermittent four weeks fasting died during the fifth week. It could probably be that their intestinal system was unable to restore a normal functioning when food was given again. Contrary to survival rate stated in this work, El-Emam and Madsen (1981) reported an increase in mortality later in 75% of *B. truncatus* that survived 7 days starvation while another species *B*. *alexandrina* survived the 7 days starvation. Similarly, Armour *et al*. (2016) reported the survival of all the *B. glabrata* at 4, 7 and 10 days period of starvation while snails starved at 14 and 20 had significant death rate. The weight of snails in this study is supported by Mohammed *et al*. (1986) who demonstrated the effect of starvation and re-feeding in *B. truncatus,* they observe a decrease in wet weight of the snails after the first 2 days of starvation, which they suggested was due to reduction of stored materials.

The result of the Thin Layer Chromatography (TLC) in the present work revealed the presence of phospholipids and fatty acids as the main lipids and additional classes of lipids (tryglycerol and wax) were seen in the starved snail *B. truncatus*. However, other researchers reported the presence of divers lipids in different species of planorbids. White *et al.* (2007) reported the presence of triacylglycerol, steryl esters, free fatty acid, free sterols and phosphatidylcholine in *B*. *glabrata* using high performance thin layer chromatography-densitometry after 7 days starvation. Similarly, The TLC results by other authors indicated that free sterols and triacylglycerols are the main lipids of both fed and starved *B. glabrata* along with lesser amount of free fatty acid and methyl esters (Duncan and Fried 1987 and Concetta *et al*., 1996). Zhukova (2014) reported that the main lipid classes of nudibranchs are phospholipid, sterol, free fatty acid, triacylglycerol and monoalkyldiacylglycerol. Rakshit *et al*. (1997) also reported the presence of phospholipid, cholesterol, and, to a lesser extent, triglycerides in the gastropod *Telescopium telescopium* inhabiting estuarine waters of West Bengal, India after TLC analysis. The observed differences in the lipid classes of these gastropods vary probably because of species specificity, environmental conditions and food availability. Thus, under physiological conditions of stress, degradation of lipids component might occur.

The mean total lipid of *B. truncatus* (phospholipids and fatty acids level) in this study declined by 56%; after 14 days food withdrawal. Duncan and Fried (1987) observed 12% decrease in the mean total lipid of starved *B. glabrata*. Following similar studies on starvation, Pinheiro (1996) observed a reduction averaging 80% in the main deposits of carbohydrates in *B. similaris* after 30 days starvation. Other authors observed degradation in the nitrogenous products in snails in response to the changes that occurred during food deprivation (Becker and Hirtrack 1975). Concetta *et al*. (1996) observed a decrease in amount of each lipid in the digestive gland, gonad complex when food was restricted in *B. glabrata* snails. Several authors recorded reduction in triacylglycerol, sterol ester and free fatty acids from planorbids snails when they were deprived from food (Duncan and Fried 1987, Armour *et al*., 2016). Likewise, White *et al*. (2006) examined starved and aestivated snails and reported a significant decrease in the triacylglcerols in the whole body of starved *B. glabrata* when compared with the control after 14 days starvation. Thompson (1987) also reported that starvation reduced the relative level of the unidentified lipid but had little effect on triglyceride.

The possibility of observed triglycerol in starved condition in the present work could be as a result of the nutritional demands in starved snails that reached to a point where wide spread metabolism occurred. One of the first changes may be the shutdown of nutrient for reproductive purposes, which later induces the breakdown of reserved carbohydrates in albumen glands to meet the demand of starvation. It follows therefore that, the reduction in energy level seen in starved snails consequently reduces the rate of fecundity which will serve as a control measure and eradication ideas. In the field during rainy season, schistosomiasis is propagated because of food and the rates of fecundity are high but during dry season, the water canals are dried up and the snails hide in the mud of void water canals. This decreases the availability of food for the snails (Mohammed *et al.*, 1986). Starvation due to a pause in irrigation activities reduces the rate of fecundity in *B. truncatus.* Therefore, activities directed towards eradication should be carried out primarily during dry periods. It is expected that repeated application of molluscicide would kill a large part of the population, while there is no population increase due to reduced reproduction.

CONCLUSION

There as an observed alteration in the lipid profile of snail (*Schistosoma* intermediate host) used in this study as a result of starvation. Since lipids utilizes important biological functions as energy storage compounds and were seen altered during withdrawal of food in this study, therefore, eradication of this snail host should be more efficient if their habitat is deprive of food.

REFERENCES

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

Armour, E., Fried, B. and Sherma, J. (2016). Effects of starvation for 4–20 days on the neutral lipid and phospholipid content of *Biomphalaria glabrata* as determined by high performance thin layer chromatography-densitometry. *Journal of Liquid Chromatography & Related Technologies* **39**:(5-6), 322-324.

Becker, W. and Hirtrack, E. (1975). Effect of Starvation on total protein and hemoglobin concentration in the hemolymph of *Biomphalaria glabrata. Journal of Comparative Biochemistry and Physiology, **51A***:15-16.

Brown, T. L. and Benjamin, J. (1964) Usefulness of thin layer chromatography technique. *Analytical Chemistry, **36***: 446-447

Brown, D.S., Oberholzer, G and Van Eeden, J. A. (1971). The *Bulinus natalensis/tropicus* complex in Southern Affrica. 1. Shell, mantle, copulation organ and chromosome number. *Malacology **11***: 141-470

Duncan, M. and Fried, B. (1987). Lipids in fed and starved *Biomphalaria glabrata* (gastropoda). *Comparative Biochemistry Physiology*- **Part A. 86A (4)** 663-665

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

El-Emam, M. A. and Madsen, H. (1981). The effect of temperature, darkness, starvation and various food types on growth, survival and reproduction of *Helisoma Duryi*, *Biomphalaria alexandrina* and *Bulinus truncatus* (Gastropoda: Planorbidae). *Hydrobiologia,* 88:265

Emejulu, A. C., Alabaronye, F.F., Ezenwaji, N.M. and Okafor, F.C. (1994). Investigation into the prevalence of urinary schistosomiasis 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.

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

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.

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.

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

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 cem, Nkalagu Eastern Nigeria: Observation on some demographic aspects of the schistosome-transmitting Bulinids. *Animal Research International, **1(2):*** 120-124.

Okafor, F.C. (1990). *Shistosoma haematobium* 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.

Pinheiro, J. (1996). influence of starvation on the glycogen contents in the snail *Bradybaena similaris* (Ferussac, 1821) (Mollusca, Gastropoda). *Arquivos Biologia e Technologia*, ***39(2):*** 349-357

Rakshit, S., Bhattacharyya, D. K., Misra, K. H. (1997). Distribution of Major Lipids and Fatty Acids of the Estuarine Gastropod Mollusc *Telescopium telescopium*. *Folia Biologica, **45 (1):*** 83-87

Remy, M. and Arouna, N. (2005). Studies on the morphology and compatibility between *Schistosoma hæmatobium* and the *Bulinus* sp. complex (gastropoda: planorbidae) in Cameroon. *African Journal of Biotechnology, **4(9):*** 1010-1016

Skipski, V.P, Smolowe, A.F, Sullivan, R.C., and Barclay M. (1965). Separation of Lipids Classes by thin layer chromatography. *Biochimica* et *Biophysica Acta*., ***106:*** 386-396.

Smith, T. M., Brooks, T. J. Jr. and White, H. B. Jr (1966). Thin layer and gas-liquid chromatography analysis of lipid from cercaria of *Schistosoma mansoni. American Journal of Tropical Medicine and Hygiene. **15,*** 307-313

Stothard, J. R., Brémond, P., Andriamaro, L., Sellin, B., Sellin E. and Rollinson, D. (2001). *Bulinus* species on

Madagascar: molecular evolution, genetic markers and compatibility with *Schistosoma haematobium*. *Parasitology,* ***123***: 261–275.

Sturrock, R. (1970). An investigation of some factors inflencing the survival of St. Lucian *Biomphalaria glabrata* deprived of water. *Annals Tropical Medicine and Parasito*logy, ***64***: 365-371

Smith, M. C., Webster, C.L., Sherma, J. and Fried, B. (1995). Determination of neutral lipids in regular and lowfat eggs by high performance TLC with densitometry. *Journal of Liquid Chromatography*, ***18***:527-535.

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

Vianey-Liaud, M., (1984). Effects of starvation on growth and reproductive apparatus of two immature freshwater snails *Biomphalaria pfeifferi* and *Biomphalaria glabrata* (Gastropoda: Planorbidae). *Hydrobiologia* ***109***: 165–172.

Vianey-Liaud, M. and Lancastre, F. (1994) Effects of intermittent starvation on mature *Biomphalaria glabrata* (Gastropoda: Planorbidae). *Hydrobiologia,* ***291(2***) 125-130

Vianey-Liaud, M. and Lancastre, F. (1986). Weight loss and survival of *Biomphalaria glabrata* deprived of water. *Mem Institute Oswaldo Cruz, Rio de Janerio*, ***81(2)***:221-224

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

White, M.M., Fried, B. and Sherma, J. (2007). Effects of Aestivation and Starvation on the Neutral Lipid and Phospholipid Content of *Biomphalaria glabrata* Infected with *Schistosoma mansoni. Journal of Parasitology*, ***93(1***):1-3.

White, M. M., Fried, B. and Sherma, J. (2006). Determination of the effects of estivation and starvation on neutral lipids and phospholipids in *Biomphalaria glabrata* (NMRI strain) and *Helisoma trivolvis* (Colorado strain) snails by quantitative high performance thin layer chromatography-densitometry. *Journal of Liquid Chromatography & Related Technologies*, ***29***:2167–2180

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

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