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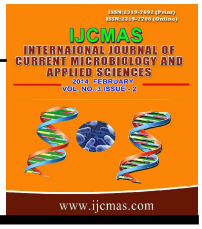


**Original Research Article**

# Studies on the effect of crude oil fractions on the lipid profile of *Achatina achatina*

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**A B S T R A C T**

Ninety snails of the genus *Achatina achatina* were collected from Nsukka environs. They were divided into Groups A, B, C and D. Groups A, B, and C were treated with crude oil fractions of petrol, diesel and kerosene respectively at concentrations of 50 ml, 75 ml and 100 ml. Group D was used as the control. The experiment was done for 28 days although total mortality occurred in Group A by the 7th day of treatment. The effects of the crude oil fractions on the lipid profile of the snails were investigated and results indicated significant changes due to the lipid concentrations. The results also showed that an increase in the crude oil fractions concentration could significantly decrease the lipid profile of *Achatina achatina.*

**Keywords**

*Achatina achatina*, lipid profile, crude oil fractions and Nsukka.

## Introduction

*Achatina is* an invertebrate animal belonging to the phylum mullusca. It is a land snail that posses a calcareous shell covering. *Achatina achatina* belongs to the family *Achatinidae* and is widely used as a source of protein in the diets of West African families including Nigeria (Bequaert, 1950*).* In Nigeria, snail meat is a cherished delicacy among the rich families and personalities. According to Yoloye (1984), snails are the largest groups of molluscs constituting the largest animal group after arthropods. The habitat of land snails ranges from the dense topical high forest in southern Nigeria to the fringing riparian forest of the derived Guinea Savanna (Ajayi et *al.,* 1978; Odaibo, 1997). *Achatina achatina* is very common in the rainforest of Nigeria and the entire West African. It is very common during the rainy season in the Western and Eastern part of Nigeria. *Achatina achatina* is nocturnal as well as diurnal. It is usually inactive during the day when they hide under the vegetation or fallen tree branches. Nigerian snails aestivate November to March because of the hot dry weather. During this aestivation period, the aperture is temporarily closed by calcified material known as epiphragm which is a whitish, fragile material (Nisbet, 1974). During this aestivation, the

snails bury themselves in soil or hide beneath stones in order to avoid direct solar radiation (Schmidt-Nielsen *et al.*, 1971). When the rain comes, epiphragm breaks and very cold water stored before aestivation pours out of the aperture (Ajayi *et al,* 1980) and the snails emerge to eat the new plant and the soft soil (Ajayi et at, 1980); Odaibo, 1997).

*Achatina achatina* is a herbivore that feeds on leaves of paw-paw *(Carica papayq),* water leaves *(Talinum triangulare),* as well as dead and rotten leaves Ajayi *et al,* 1978; Marigornez and Sacz, 1985; Egonmwan, 1988) However, it has also been reported that the animal feed on grains like guinea corn (Amusan, 1990) and other vegetables that man use as food. Besides being edible, *Achatina achatina* is economically important as the shells are used for decoration and ornamentation.

The studies on the nutritive value of snail meats have produced useful information that supports the consumption of snail meat. For example, several studies have shown that the fat content of snail is lower than that of mammalian or poultry flesh. It is rich in calcium with an exceptionally high content of iron which measures up to 12.2mg/l00g. The calorific value of snail meat is about 80k / cal/100g.

Lipids are chemically diverse group of biological substances made up of non-polar groups. They are soluble organic substances that can only be extracted from cell by organic solvents. Basically, there are three classes of lipids;

The neutral lipid
The phospholipids, and
The sterols.

These lipids play important role in human nutrition. Fats and oil are the principal stored form of energy in many organisms. Phospholipids and sterols are the major structural element of biological membranes. Other lipids, although present in relatively small quantities play crucial role as enzyme co-factors, electron carriers, high absorbing pigments, hormones and intracellular messengers. Lipids have high calorific values than carbohydrates. Fat deposits in the body serve as insulation and provides protection cushion for the organs. Essential fatty acids are important in the maintenance of normal skin condition (Burr and Burr, 1930).

Lipids are important dietary constituents not only because of their high energy values but also because of the fat soluble vitamins and the essential fatty acids contained in the fat of natural foods (Collis *et al.,* 1996). In the body, *fat* serves as an efficient source of energy, both directly and potentially when stored in adipose tissue. It serves as thermal insulators and allows rapid propagation of wave depolarization along myelinated nerves.

The fat content of nerve tissue is particularly high. Combinations of fat and protein (lipoproteins) are important cellular constituents occurring both in the cell membrane and also in the mitochondria within the cytoplasm, and serves also as the means of transporting lipids in the blood. A knowledge of lipid biochemistry is important in understanding biomedical areas of interest, example; obesity, atherosclerosis and the role of various polyunsaturated fatty acids in nutrition and health (Gurr and Harwood, 1991).

The objective of this research was to investigate the effects of different

concentrations of crude oil fractions of petrol, diesel and kerosene on the lipid profile parameters of triglycerides, high density lipoprotein, low density lipoprotein and total cholesterol concentrations of *Achatina achatina* over a period of 28 days.

## Materials and Methods

Determination of Total cholesterol concentration was by the method of Zlatkis *et al.*, (1953), which is based on the reaction of both cholesterol and cholesteryl esters with a solution of ferric chloride in a mixture of glacial acetic acid and concentrated sulphuric acid. 0.5 ml of the sample was pipetted into a test tube while 0.5 ml of distilled water was also added to a second test tube for the blank. 3ml of glacial acetic acid was added into the test tubes which were mixed thoroughly by stirring with a glass rod. These were allowed to cool at room temperature after which 2ml of reagent solution (i.e. iron stock) was added to each of the test tubes and mixed again thoroughly by stirring rod.

Colour changes from brown to purple within two minutes were noticed. This was allowed to stand for 1 hour at room temperature and the absorbance of each sample was measured with spectrophotometer at 560 nm (i.e. the wavelength).

### Determination of Total Triglyceride (TG)

The total triglyceride (TG) was determined by the Carison (1963) method. 1ml of the sample was pipetted into a flat bottom tube with 5ml of methanol. This mixture was swirled until slurry was formed. 10ml of chloroform was added to the slurry formed together with 15 ml of saline solution. This was stirred thoroughly until aqueous and chloroform layers separated. 8ml of the chloroform layer, which contained the lipid, was pipetted with the help of a syringe. The tube was closed and allowed to stand for 2 minutes and centrifuged for some minutes. 0.5ml of the clear chloroform was pipetted into test tubes with 0.5 ml of tripalmin, which served as the standard. To another tube that served as blank 0.5 ml of chloroform was poured. The whole tubes (i.e. sample, standard and blank) were placed in a water bath until the chloroform was evaporated. 1ml of ethano1 potassium hydroxide was added to each of the tubes with repeated heating for 3 minutes in a water bath. After cooling to room temperature, 0.5 ml of concentrated sulfuric acid and 4 ml of diethylether were added to the tubes. This was shaken vigorously for 30 seconds and allowed to stand for 1 minute, after which the upper layer was discarded. 0.3 ml of the remaining solution was pipetted into test tubes and 20 ml of sodium peroxide solution was added to each of the tubes. The 25 ml of chromotropic acid reagent was added and the tubes were kept in a bath of boiling water for 30 minutes. After cooking, the concentrations of triglyceride (TG) in each of the tubes were measured at 570 nm using spectrophotometer.

### Determination of Total Lipids

The total lipid was determined using the method of Zoilner and Kirsch (1962). 0.1ml of each sample was pipetted into test tubes containing 2ml of concentrated sulfuric acid. The tubes were carefully swirled and placed in a bath of boiling water for 10 minutes. The sample was allowed to cool. 0.1ml of this mixture was pipetted and transferred into a different set of tests tubes containing 2.5 ml of

phosphoric acid (Vanillin reagent) and was carefully mixed. After 30 minutes, the readings were taken at 530nm using spectrophotometer.

## Results and Discussion

### Lipid parameters of groups B and C

### Triglyceride (TGC)

On day 14 of treatment, TGC of Group B (diesel) varied from 89.50±0.14 (l00ml) to 211.10±0.14 (0ml) as shown in (Table1). Also the TGC of *Achatina* in Group C (Kerosene) varied from 40.10 ± 0.14 (100ml) to 211.10±0.14 (50ml) as shown in (Table II). TGC decreased with an increase in the concentration of group C. (Kerosene).

On day 28 of experiment, TGC of Group B varied from 72.05 ± 0.07 (100 ml) to 200.00 ±0.00 (0 ml) in (Table 1). Also the TGC in Group C ranged from 28.10 ± 0.14 (100 ml) to 200.00 ± 000 in (Table II). In both Groups, TGC were decreasing with an increase in concentration. Group C was found to have more effect across the groups after the $14^{th}$ and $28^{th}$ day of the experiment as shown in (Table IV).

### High Density Lipid (HDL)

On day 14 of the experiment, HDL of Group B varied from a minimum of 26.35 ± 0.07 (50 ml) to a maximum of 70.00 ± 0.07 (0 ml) in (Table I). Also the HDL of Group C ranged from 20.50 ± 0,14 (50 ml) to 93.15 ±0.07 (0 ml) as shown in (Table II).

On day 28 of the experiment, the HDL of Group B varied from a minimum of 15.75 ± 0.07 (50 ml) to a maximum of 93.15 ± 0.07 (0 ml) in (Table I) while HDL of group C also varied from a minimum of 20.50 ± 0.14 (50 ml) to a maximum of 93.15 ± 0.07 (0 ml) as shown in (Table II). Finally on day 14 of experiment, Group C had more effects across the groups while Group B had more effects on day 28 across the groups.

### Low Density Lipid (LDL)

On day 14 of the experiment, the LDL of Group B ranged from a minimum of 39.55 ± 0.07 *(*50 ml) to a maximum of 139.30 ± 0.14 (0 ml) in (Table I). The LDL of Group C varied from a minimum of 126.25 ± 0.07 (50 ml) to a maximum of 139.30 ± 0.14 (0 ml) as shown in (Table II). On day 28 of the experiment, the LDL of Group B ranged from 35.25 ±0.07 (100 ml) to 101.35 ± 0.07 (0ml) in (Table I). The LDL of group C varied from a minimum of 50.40 ± 0.14 (100 ml) to a maximum of 114.25 ± 0.07 (50 ml) as shown in (Table II). Also, on day 28 of the experiment, the LDL concentration decreased with an increasing concentration in both groups B and C. Finally, Group C was found to have more effects across the groups on day 14 while Group B was found to have more effects on day 28 across the groups as shown in (Table IV).

### Total Cholesterol (TC)

On day 14 of experiment, The TC f Group B ranged from 115.85 ± 0.07 (50 ml) to 252.55 ±0.07 (75 ml) in (Table 1). The TC of Group C ranged from a minimum of 94.65 ± 0.07 (100 ml) to a maximum of 209.65 ±0.07 (0ml) in (Table II). It was found that in Group C, the TC was decreasing with an increase in concentration of kerosene. On day 28 of the experiment, TC of Group B varied from a minimum of 70.00 ± 0.00 (100 ml) to a maximum of 194.50 ±0.14 (0 ml) in (Table I). Also, the TC of Group C varied

from a minimum of 80.85 ± 0.21(100 ml) to a maximum of 194.50 ± 0.14 (0 ml) in (Table II). It was found that after 28 days of treatment, the TC was decreasing with an increasing concentration of Groups B and C. Finally, on day 14 of the experiment, Group C had more effects across the groups while Group B had more effects across the groups on day 28 of the experiments (Table IV).

**Lipid parameters for group A**

**Triglyceride (TGC)**

On day 7 of treatment, the TGC of Group A ranged from 27.10 ±0.14 (100 ml) to 117.10 ± 0.14 (0ml) in (Table III).

**High Density Lipid (HDL)**

On day 7 of treatment, the HDL of Group A varied from a minimum of 13.80±0.14 (100 ml) to 62.60 ± 0.28 (0 ml) in (Table III).

**Low Density Lipid (LDL)**

On day 7 of treatment, the LDL of Group A ranged from 25.40 ± 0.28 (50 ml) to 160.85 ± 0.07 (75 ml) in (Table III).

**Total Cholesterol (TC)**

On day 7 of treatment, TC of Group A varied from a minimum of 46.25 ± 0.35 (50 ml) to a maximum 186.00 ± 0.00 (75 ml) as shown in (Table III).

According to Okolie (1985), lipids are an integral components of the cellular membrane of a living organism. Nwadinigwe (1981) found that the amount of fat in *Achatina achatina* varies from 5,11 — 0.74% of dry weight.

The results of this research indicated that diesel, kerosene and petrol could ($p<0.05$) significantly change the concentration of lipids in an animal. This is in line with the work of Nechev *et al,* (2002) involving the effect of diesel fuel treatment on the lipid profile of snail *Rapana thomasiana,* the diesel fuel treatment significantly changed the fatty acid composition.

This research showed that *Achatina* is a good source of lipid. Petroleum contamination of the environment has been recognized as a serious pollution problem, this research has shown that petroleum compounds like petrol, diesel and kerosene has a reduction effect on the lipid concentration *of Achatina.* This also collaborated with the observation that some marine gastropods could accumulate short-chain aliphatic hydrocarbon in their tissues (Walsh *et al.,* 1995). The effects of these hydrocarbons on the tissue and general lipid profile of the snail could also vary, from being more lethal i.e. petrol which caused mortality in less than 7 days to being mild in the case of diesel and kerosene where the animals lasted even beyond 28 days. The question of how exactly the hydrocarbon exert their effects on the concentration of lipids of this animal is still subject to further research.

**Table.1** The concentration of lipid parameters for Group B (Diesel).
Concentration

| Days | Parameters | 0 ml | 50 ml | 75 ml | 100 ml |
|---|---|---|---|---|---|
| 14 Days | Triglyceride | 211.10±0.14 | 90.50±0.07 | 199.50±0.07 | 89.50±0.07 |
| | HDL | 70.00±0.07 | 26.35±0.07 | 63.66±0.07 | 52.70±0.14 |
| | LDL | 139.30±0.14 | 39.55±0.07 | 189.10.0.14 | 52.50±0.14 |
| | TC | 209.65±0.07 | 115.85±0.07 | 252.55±0.07 | 131.80±0.28 |
| 28 Days | TGC | 200.00±0.00 | 75.05±0.07 | 74.15±0.21 | 72.05±0.07 |
| | HDL | 93.15±0.07 | 15.75±0.07 | 36.35±0.07 | 34.75±0.07 |
| | LDL | 101.35±0.07 | 74.30±0.00 | 39.65±0.07 | 35.25±0.07 |
| | TC | 194.50±0.14 | 70.05±0.07 | 75.50±0.71 | 70.00±0.00 |

**Table.2** The concentration of Lipid Parameters for Group C.
Concentration

| | **Parameters** | **0 ml** | **50 ml** | **75 ml** | **100 ml** |
|---|---|---|---|---|---|
| 14 Days | TGC | 211.10±0.14 | 150.10±0.14 | 50.15±0.21 | 40.10±0.14 |
| | HDL | 70.00±0.07 | 31.70±0.14 | 52.65±0.07 | 39.45±0.07 |
| | LDL | 139.30±0.14 | 126.25±0.07 | 94.55±0.21 | 55.50±0.14 |
| | TC | 209.65±0.07 | 157.95±0.07 | 147.70±0.42 | 94.65±0.07 |
| 28 Days | TGC | 200.00±0.00 | 126.10±0.14 | 36.10±0.14 | 28.10±0.14 |
| | HDL | 93.15±0.07 | 20.50±0.14 | 40.20±0.14 | 30.45±0.07 |
| | LDL | 101.35±0.07 | 114.25±0.07 | 87.50±0.28 | 50.40±0.14 |
| | TC | 194.50±0.14 | 134.75±0.14 | 127.70±0.42 | 80.85±0.21 |

**Table.3** The concentration of Lipid Parameters for Group A
Concentration

| | **Parameters** | **0 ml** | **50 ml** | **75 ml** | **100 ml** |
|---|---|---|---|---|---|
| 7 Days | TGC | 117.10±0.14 | 55.10±0.14 | 80.05±0.07 | 27.10±0.14 |
| | HDL | 62.60±0.28 | 20.85±0.07 | 25.15±0.07 | 613.80±0.28 |
| | LDL | 48.45±0.21 | 25.40±0.28 | 160.85±0.07 | 48.45±0.21 |
| | TC | 111.15±0.64 | 46.25±0.35 | 186.00±0.00 | 111.15±0.64 |

**Table.4** The concentration of Lipid Parameters Across the Groups

| | **Parameters** | **Group B** | **Group C** | **Control** |
|---|---|---|---|---|
| 14 Days | TGC | 126.50±56.55 | 80.12±54.40 | 211.10±0.12 |
| | HDL | 47.57±17.15 | 41.27±9.47 | 70.35±0.05 |
| | LDL | 110.38±63.19 | 92.02±31.81 | 139.30±0.12 |
| | TC | 166.73±77.86 | 133.43±30.39 | 209.65±0.55 |
| 28 Days | TGC | 73.75±1.38 | 63.43±16.99 | 200.00±0.00 |
| | HDL | 28.95±10.25 | 30.38±8.81 | 93.15±0.07 |
| | LDL | 49.73±19.13 | 114.43±26.20 | 194.50±0.14 |
| | TC | 78.51±9.27 | 114.43±26.20 | 194.50±0.14 |

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