Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 7 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Lipid Composition of Three Different Types of Land Snails Consumed in Nigeria

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GJSFR-B Classification : *FOR Code: 030499*

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I. Introduction

The conventional sources of meat protein for the Nigerian populace come mainly from livestock in form of poultry, beef, mutton and pork. These traditional sources are being faced by certain constraints such as the persistent and severe sahelian drought, diseases, high cost of feed, primitive animal husbandry techniques and the low productivity of local animal breeds. The rapid growth of human population (Oyenuga, 1968) together with the rising standard of living has also placed great pressure on the existing sources of animal protein.

The constraints earlier enumerated limit radical increase in the domestic livestock production in Nigeria; hence other non-conventional sources of protein are being investigated. Land snails are non-conventional wildlife protein source. Human consumption of the land snails has been practised since the very earliest times. The main users are at present the populations of West Africa and West Europe and their markets are supplied, mainly, with wild snails. In Nigeria, the edible land snails are fast becoming culinary delicacies (referred to as "Congo meat") and demand has been so great that snail farming is gaining importance (Odaibo, 1997). The snail represents food of high nutritive value with a shell mainly composed of calcium carbonate, and flesh consisting of water (at least 70 %) and protein (about 60-70 % on dry basis). The giant snails are rich in lysine and generally low in cholesterol.

Snails now constitute an important source of animal protein for many coastal communities in Nigeria and Ghana. Marine and terrestrial gastropods collected for food include the following species (Yoloye, 1984):

1. The abalome – *Maloitis tuberculata*.
2. The large Ghana cowrie- *Cypraea stecocoratia.*
3. The rock limpet-*Patella safiana*.
4. The lagoon whelk-*Semifuscus morio*.
5. The lined mangrove periwinkle- *Littorina angulifera*.
6. *Thais califera* and *Thais haemastoma* (the dog whelk).
7. The large volute-*Cymbium cymbium*.
8. The Nigeria garden snail (Ipere) *Limicolaria* sp.
9. The giant African land snail-*Archachatina marginata.*
10. The mangrove mud periwinkle-*Tympanotonus fuscutus.*
11. *Archatina* sp. (Ilako).

In West Africa, particularly in Nigeria, various species of *Archatina* and *Archachatina* are eaten to a great extent. In some cases they actually form the largest single item of animal protein in the diet of the common people in rural areas (Odaibo, 1997).

Few publications are available on the nutritional qualities of Nigeria land snails. Published works include Odaibo (1997) on snail and snail farming; Cooper and Knowler (1991) on snails and snail farming (an introduction); Adeyeye (1996) on waste yield, proximate and mineral composition of three different types of land snails found in Nigeria; Adeyeye (1998) on the mineral composition of the haemolymph of three different types of land snails consumed in Nigeria; and Adeyeye and Afolabi (2004) on the amino acid composition of three different types of land snails consumed in Nigeria. The study in this paper is therefore, an attempt to assess the lipid concentration (crude fat, fatty acids, phospholipids and zoosterols) from land snails consumed in the Southwest zone of Nigeria. These are the Nigeria garden snail (*Ipere*) *Limicolaria* sp.; (*Ilako*) *Archatina (archatina) archatina* (Linne) and the giant African land snail, *Archachatina (Calachatina) marginata* (Swaison).

II. Resources and Techniques

a) Materials

Snail samples were collected from the farm at Odo Ayedun-Ekiti, Ekiti State, Nigeria. The samples were

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collected in the month of June, 2011. Samples were then identified.

Samples were washed with distilled water and then wrapped separately in aluminium foil and frozen at -4°C for 5 days before samples were prepared for analysis.

The shells were carefully removed to recover the edible parts. The edible parts were cut into small pieces and dried at 95-105 °C until dried and ground into fine powder.

b) Determination of ether extract

An aliquot (0.25 g) of each part was weighed in an extraction thimble and 200 ml of petroleum ether (40 -60 °C boiling range) was added. The covered porous thimble containing the sample was extracted for 5 h using a Soxhlet extractor. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether, oven dried at 50 °C for 1 h, cooled in a desiccator and the weight of dried oil was determined (AOAC, 2005). Determinations were in duplicate.

c) Preparation of fatty acid methyl esters and analysis

A 50 mg aliquot of the dried oil was saponified for 5 min at 95 °C with 3.4 ml of 0.5 MKOH in dry methanol. The mixture was neutralized by 0.7 MHCl and 3 ml of 14 % boron triflouride in methanol was added. The mixture was heated for 5 min at 90 °C to achieve complete methylation. The fatty acid methyl esters were thrice extracted from the mixture with redistilled *n*-hexane and concentrated to 1 ml for analysis. The fatty acid methyl esters were analysed using an HP 5890 gas chromatograph (GMI, Inc., Minnesota, USA) fitted with a flame ionization detector and using ChemStation software. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven programme was: initial temperature at 60 °C, ramping at 10 °C/min for 20 min, held for 4 min, with a second ramping at 15 °C/min for 4 min and held for 10 min. The injection temperature was 250 °C and the detector temperature was 320 °C. A capillary column 30 m x 0.25 mm was packed with a polar compound (HP INNOWAX) onto a diameter of 0.25 μm was used to separate the esters. A split injection was used with a split ratio of 20:1. The peaks were identified by their relative retention time compared with known standards (AOAC, 2005). Determinations were in duplicate.

d) Sterols analysis

Aliquots of the dried oil were added to screw-capped test tubes. The samples were saponified at 95 °C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 ml of benzene was added to ensure miscibility. Deionised water (3 ml) was added and 2 ml of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 ml of hexane, were carried out for 1 h, 30 min and 30 min respectively, to achieve complete extraction of the sterols. Hexane was concentrated to 1 ml for gas chromatographic analysis (AOAC, 2005). Determinations were in duplicate.

e) Phospholipids analysis

Using a modified method of Raheja *et al.* (1973), 0.01 g of the dried oil was added to test tubes. Any remaining solvent was removed by passing a stream of nitrogen gas over the oil. Then 0.40 ml of chloroform was added, followed by addition of 0.10 ml of the chromogenic solution. The tube was heated to 100 °C in a water bath for 1 min 20 sec, cooled to room temperature, 5 ml of hexane was added and the tube was shaken gently several times. After separation of the solvent and aqueous layers, the hexane layer was recovered and concentrated to 1.0 ml for analysis. Analysis was performed using the gas chromatograph with a capillary column 30 m x 0.25 mm packed with a polar compound (HP 5) onto a diameter 0.25 μm. The oven programme was: initially at 50 °C, ramping at 10 °C/min for 20 min, held for 4 min, a second ramping at 15 °C/min for 4 min and held for 5 min. The injection temperature was 250 °C, and the detector temperature was 320 °C. As previously described, a split injection type was used having a split ratio of 20:1. Peaks were identified by comparison with the known standards. Determinations were in duplicate.

f) Quality assurance

Standard chromatograms were prepared for sterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient was determined for each fatty acid (34), sterol (7) and phospholipid (5). Correlation coefficient $>$ 0.95 was considered acceptable

g) Statistical analysis

Statistical analysis (Oloyo, 2001) was carried out to determine the mean, standard deviation, coefficient of variation in per cent. Also calculated were the chi-square (X^2) values. The X^2 was subjected to the table (critical) value at $\alpha = 0.05$ to see if significant differences existed in the values of fatty acids, sterols and phospholipids between the snail samples.

III. Results

a) Fatty acids

In Table 1, the crude fat varied between 2.22 g/100 g to 2.38 g/100 g. The values were close with the coefficient of variation per cent (CV %) being low at 3.67. The total energy coming from the crude fat was also low at 82.1-88.1 kJ/100 g.

Table 2 contains the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) values. The following acids under this section were not detected:

C2:0, C3:0, C4:0 (in *Archachatina marginata)* and *Archatina archatina,* C6:0, C8:0, C12:0 in *A. marginata* and *Limicalaria* sp. whereas the following fatty acids (FAs) recorded 0.00 % of the total fatty acids: C5:0, C20:0, C24:0, C22:*1 cis*-13 and C24:*1 cis-15.* Low levels of C4:0, C10:0, C12:0, C14:0, C22:0, C14:*1 cis-9*, C16:*1 cis*-9 and C20:*1 cis-11* with each of their value being less than 1.0 % of total fatty acids. It is however interesting to observe that most of their CV % values were mostly 37.2-37.7 with only one (C14:*1 cis-9*) having a CV % of 26.7. Among the SFA, C18:0 was the most concentrated in all the samples and had a range of 23.4-28.7 % total FA with CV % of 10.2. This was closely followed by C16:0 with values of 13.6-19.4 % and CV % of 17.5. For the two FAs the trend of concentration was *Limicolaria* sp > *A. marginata* > *Archatina archatina*. Total SFA range was 49.8 % (*Limicolaria* sp) > 43.0 % (*A.marginata*) > 37.5 % (*Archatina archatina*). Among C18:*1 cis* MUFA, C18:1 *cis*-6 was the most concentrated with values of 2.43-3.41 % and CV % of 17.8 whereas C18: 1*cis*-9 followed with values of 2.41-6.11 % but higher CV % of 57.1. The best source of C18:1 *cis*-9 was in *Limicolaria* sp. (6.11 %). Total MUFA (*cis*) range was 6.11-9.15 % and CV % of 22.4. All the C18:1 *trans* levels were mostly higher than the C18:*1 cis* but with lower levels of CV %. C18:*1 trans*-6 had a range of 6.35-8.44 % and CV % of 15.4; in C18:1 *trans*-9, range was 5.08-6.07 % and CV % of 9.30; and C18:1 *trans*-11 being 3.17-3.94 % and CV % of 11.4. Total C18:1 *trans* was 15.6-17.4 % with CV % of 6.72. The total MUFA values had *a* distribution of 24.8 % (*Limicolaria* sp.) > 24.0 % (*A. marginata*) > 23.8 % (*Archatina archatina*) and low CV % of 2.19.

In Table 3, PUFA *n*-6 and *n*-3 FA composition of the three snail samples were depicted. The following *n-6* PUFA had 0.00 % total fatty acids observed for them: C20:3 *cis*-8, 11, 14, C20:4 *cis*-5, 8, 11, 14 and C22:2 *cis*-13, 16; for the *n*-3 PUFA, the following were in similar category: C20:3 *cis*-11, 14, 17 and C22:6 *cis*-4, 7, 10, 13, 16, 19. Three prominent *n*-6 FAs were observed in the three samples. The first and mostly concentrated in all the samples was C20: 2 *cis*-11, 14 with value range of 8.36-16.7 % and CV % of 33.2. The next highest *n-6* FA was C18:2 *cis*-9, 12 with a range of 7.30-9.51 % and CV % of 13.2. C18: 3 *cis*-6, 9, 12 was low in value with a range of 1.07-1.27 % but least CV % (8.67) among the three. Total *n-6* PUFA (*cis*) was 27.4 % (*Archatina archatina*)> 22.6 % (*A. marginata*) > 16.7 % (*Limicolaria* sp.) with CV % of 24.1. The only *n*-6 but important PUFA *trans* was C18:2 *cis*-9, *trans*-11 (rumenic acid) with comparable levels as C18:2 *cis-9, 12*; rumenic acid had a range of 7.37-9.79 % with CV % of 14.1. Total *n-6* PUFA was 37.2 % (*Archatina archatina*) > 31.4 % (*A. marginata*) > 24.1 % (*Limicolaria* sp.) with CV % of 21.2. The only *n*-3 PUFA having values greater than 0.00 % was C18:3 *cis* -9, 12, 15 with values of 1.37-1.65 % and CV % of 9.28. The values of C18:3 *cis-9*, 12, 15 were much lower than the values of C18: 2 *cis* -9, 12, both are essential fatty acids. The total *n*-6 + *n*-3 PUFA values were 38.7 % (*Archatina archatinna*) > 33.1 (*A. marginata*) > 25.5 % *(Limicolaria* sp.) with CV % of 20.4. Thus the total MUFA +PUFA range of 50.3 -62.5 % showed that the three snails were mostly composed of unsaturated fatty acids in their lipids composition.

The summary of the statistical results from Tables 2 and 3 is shown in Table 4. The X^2 results showed that no significant differences existed among the samples between their SFA, MUFA, DUFA and TUFA values. However, such results within a particular snail sample were highly and positively significantly different at $\alpha = 0.05$ since the result values of 30.9-45.1 were all higher (individually) than the critical table value of 7.82.

b) Phospholipids

Phospholipids level (mg/100 g) of the samples are in Table 5. The overall values were generally low at 1.55-2.88 mg/100 g (dry weight) and CV % of 31.3. Only lecithin had values greater than 1.00 mg/100 g in two samples and the percentage concentration ranged from 57.5-61.8 % and CV % of 29.1. Cephalin ranged between 0.226 mg/100 g and 0.450 mg/100 g and it was the second highest phospholipid. Table 5 results were subjected to X^2 analysis as shown in Table 6. At the row and vertical levels, no significant difference was observed at $\alpha = 0.05$.

c) Sterols

Table 7 depicted the sterols level (mg/100 g) of the samples. Only cholesterol was having values greater than 0.00 % in the samples. Even the cholesterol levels were low at 37.1-45.1 mg/100 g and close at 9.86 % coefficient of variation. Their X^2 value was also much less (0.805) than the critical value of 5.99 at $\alpha = 0.05$ thereby making the results not significantly different among the samples.

IV. Discussion

a) Fatty acids

The crude fat levels of 2.22-2.38 g/100 g in Table 1 were found to be much lower than other animal protein sources found in literature. Some literature crude fat levels were: 67 % (beef fat), 72 % (lamb fat), 71 % (pork fat), duck meat and skin (43 %), calf liver (7 %), chicken, meat and skin (18 %) (Bender, 1992). However the snails crude fat levels compared favourably with what obtains in the skin of *Oreochromis niloticus* fish having a value of 2.25 g/100 g (dry weight) (Adeyeye, 2011) but better than the muscle of *O. niloticus* having a value of 0.228 g/100 g; and also greater than the crude fat levels of the body of Tongue sole fish with: 0.360 g/100 g (skin) and 0.027 g/100 g (muscle) (Adeyeye *et al.*, 2011). The calculated energy from the crude fat gave values of 82.1-88.1 kJ/100 g. For somebody that requires 2500 daily calories and 15 % coming from fat oil consumption, this translates to 41.6 g of fat per day.

From the present report, a person for optimum weight loss, may reduce the overall fat/oil consumption by eating snail meat. The crude fat levels in the snails showed that each would almost supply equal levels of crude fat and energy as shown by their low CV %.

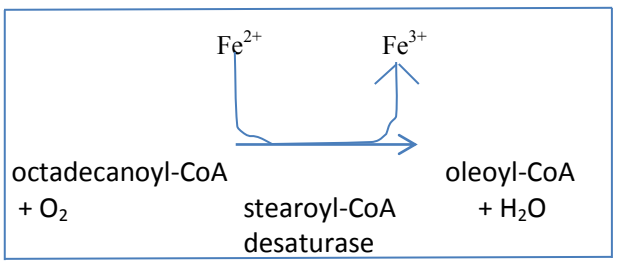
Among the short-chain fatty acids in the samples is the C4:0. It constituted just 1.09 % total fatty acid in the *Limicolaria* sp. It is mostly found in butterfat from cows. This fatty acid has antimicrobial properties- that is; it protects us from viruses, yeasts and pathogenic bacteria in the gut. They do not need to be acted on by the bile salts but are directly absorbed for quick energy. For this reason, they are less likely to cause weight gain than olive oil or commercial vegetable oils (Portillo *et al*., 1998). Short- chain fatty acids also contribute to the health of the immune system (Kabara, 1978). Medium – chain fatty acids have eight to twelve carbon atoms and are common in butterfat and the tropical oils. In the present samples C10:0 and C12:0 were present in minor quantities in the samples. Like the short-chain fatty acids, these fats have antimicrobial properties; are absorbed directly for quick energy; and contribute to the health of the immune system.

Long-chain fatty acids have from 14 to 18 carbon atoms and can be either saturated, monounsaturated or polyunsaturated. Myristic acid (14:0) is a ubiquitous component of lipids in most living organisms, but usually at levels of 1-2 % only. In the present samples C14:0 ranged from 0.116-0.256. However, it is more abundant in cow's milk fat, some fish oils and in those seed oils enriched in medium-chain fatty acids (e.g. coconut and palm kernel). In *O. niloticus* fish C14:0 formed 6.59 % FA in the skin and 4.19 % in the muscle (Adeyeye, 2011) whereas it was 1.12 % (skin) and 1.05 % (muscle) of Tongue sole fish (Adeyeye *et al*., 2011). This fatty acid is found very specifically in certain proteolipids, where it is linked via an amide bond to an N-terminal glycine residue, and is essential to the function of the protein components. Palmitic acid (16:0) is usually considered the most abundant SFA in nature, and it is found in appreciable amounts in the lipids of animals, plants and lower organisms. It comprices 20-30 % of the lipids in most animal tissues, and it is present in amounts that vary from 10 to 40 % in seed oils. The present results are at variance with these earlier observations. Here it comprised of 13.6 – 19.4 % and it is second to stearic acid. Stearic acid (18:0) is the second most abundant SFA in nature, and again it is found in the lipids of most living organisms. In these samples (18:0) occupied the highest position (23.4-28.7 %) in the SFA group. In lipids of some commercial importance, it occurs in the highest concentrations in ruminant fats (milk fat and tallow) or in vegetables oils such as cocoa butter, and in industrially hydrogenated fats. It can comprise 80 % of the total fatty acids in gangliosides. The other SFA present in minor level was behenic acid (C22:0), a member of the very-long-chain fatty acid. The total SFA of 37.5-49.8 % could easily compare favourably with literature values; they are: 43 % (beef fat), 50 % (lamb fat), 37 % (pork fat), 33 % (chicken, meat and skin), 27 % (duck, meat and skin), 30 % (calf liver) (Bender, 1992).

Oleic acid [9*c*-18:1 or 18:1(*n*-9)] is by far the most abundant monoenoic fatty acid in plant and animal tissue, both in structural lipids and in depot fats. It comprised 6.11 % of *Limicolaria* sp. FA being the highest of the *cis*-MUFA. Olive oil contains up to 78 % of oleic acid, and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet. It has a number of important biological properties, both in the free and esterified form. Oleic acid is the biosynthetic precursor of a family of fatty acid with the (*n*-9) terminal structure and with chain-lengths of 20-24 or more. Petroselinic acid (6*c*-18:1) occurs up to a level of 50 % or more in seed oils of the Umbelliferae family, including carrot, parsley and coriander. In the present report, petroselinic acid occupied the highest position in the *cis*-18:1 FA in both *A. marginata* (3.41 %) and *Archatina archatina* (3.33 %) but second highest position in *Limicolaria* sp. (3.06 %). These values are close having a CV % of 17.8.

Trans-18:1 of reasonable levels were *trans* petroselinic acid (C18:1 *trans*-6) (6.35-8.44 %), elaidic acid (C18:*1 trans-9*) (5.08-6.07 %), vaccenic acid (C18:1 *trans*-11) (3.17-3.94 %). Tissues of ruminant animals, such as cows, sheep and goats, can contain a number of different 18:1 isomers like: C18:1 *trans-9* (5.0 %) and C18:1 *cis*-9 (85 %), C18:1 *trans*-11 (47 %) and C18:1 *cis*-11 (47 %) (Hay and Morrison, 1973) with the *cis*-isomers, 9- and 11-18:1 slightly predominate as might be expected. 11*t*-18:1 makes up 50 % of *trans*-monoenes in ruminant animal tissues (which can comprise 10-15 % of the total monoenes or 3-4 % of the total fatty acids). In the present report C18:1 *trans*-11 had a range of 3.17-3.94 % of the total fatty acids and 20.3-22.3 % of the *trans*-monoenes or 12.8-16.6 % of the total monoenes. *cis*-vaccenic acid [11*c*-18:1 or 18:1 (n-7) is a common monoenoic fatty acid of bacterial lipids, and it is usually present but as a minor component of plant and animal tissues. It is occasionally a more abundant constituent of plants, for example those containing appreciable amounts of its biosynthetic precursor, 9-16:1 (e.g. the fruit of sea buckthorn). Note that vaccenic acid per se is the *trans* isomer. 11-*cis*-Eicosenoic acid [11-20:1 or 20:1 (*n*-9), gondoic] is a common if minor constitutent of animal tissues and fish oils, often accompanied by the 13-isomer. It is also found in rapeseed oil and seed oils of related species. It occupied a level of 0.069-0.152 % in the present snail samples.

In nearly all higher organisms, including many bacteria, yeasts, algae, plants and animals, double bonds are introduced into fatty acids by an aerobic mechanism that utilizes preformed fatty acids as the substrate. Molecular oxygen and a reduced pyridine nucleotide (NADH or NADPH) are required cofactors. Thus in animals and yeasts, the coenzyme A ester of octadecanoic (stearic) acid is converted directly to oleoyl-CoA by a concerted removal of hydrogen atoms from carbons 9 and 10 (D-stereochemistry in each instance). The stearoyl-CoA desaturase system is in the endoplasmic reticulum membrane with the active centre exposed to the cytosol, and consists of three proteins, cytochrome b5 reductase, cytochrome b5, and the desaturase, which contains two atoms of iron at the active site.



Membrane – bound enzymes are notoriously difficult to purify, but the evidence suggests that the yeast Δ9 desaturase consists of two membrane spanning regions with the bulk of the protein protruding into the cytosol. The enzyme has much in common with hydroxylases and contains eight essential histidine residues that coordinate with the di-iron centre at the active site. The cytochrome b5 component is fused to the desaturate and is believed to facilitate electron transfer from NADH reductase to the catalytic di-iron core.

Palmitoleate is synthesised from palmitate by a similar mechanism. Subsequently, oleate can be chain elongated by two carbon atoms to give longer-chain fatty acids of the (*n*-9) family, while palmitoleate is the precursor of the (*n*-7) family of fatty acids. In mammalian systems the elongases are known to be distinct enzymes that differ from those involved in the production of longer-chain polyunsaturated fatty acids. *Alpha*- and *beta*-oxidation can also occur to give shorter chain components of the two families.

9-18:1 →	11-20:1 →	13-22:1 →	15-24:1 →	etc.
18:1(*n*-9)	20:1(*n*-9)	22:1(*n*-9)	24:1(*n*-9)	
9-16:1 →	11-18:1 →	13-20:1 →	15-22:1 →	etc.
16:1(*n*-7)	18:1(*n*-7)	20:1(*n*-7)	22:1(*n*-7)	

Petroselinic acid (6-18:1) in seed oils of the Unbelliferae is synthesised by an enzyme that removes hydrogens from position 4 of palmitate, before the resulting 4-16:1 is elongated by two carbon atoms.

16:0 desaturation → 4-16:1 elongation → 6-18:1 petroselinic acid

The relative proportion of SFA/MUFA is an important aspect of phospholipid compositions and changes to this ratio have been claimed to have effects on such disease states as cardiovascular disease, obesity, diabetes, neuropathological conditions and cancer. For example, they have been shown to have cyto-protective actions in pancreatic β-cells. *cis*-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. They are now recognised by nutritionists as being beneficial in the human diet.

Current nutritional thinking appears to be that dietary *trans*-monoenoic fatty acids, both from ruminant fats and from industrial hydrogenation processes, should be considered as potentially harmful and in the same light as saturated fatty acids.

In Table 3, the five important long-chain and very –long-chain fatty acids were C18:2 *cis*-9, 12, C18: *3 cis-6, 9, 12*, C 18:*2 cis-9, trans-11*, C18:*3 cis-9, 12, 15* (all in the group of long-chain FAs) and C20:*2 cis-11, 14* (under very-long-chain FAs). The two essential fatty acids are C18:*2 cis-9, 12* and C18:*3 cis-9, 12, 15* with respective values of 7.30-9.51 % and 1.37-1.65 %. Another important long-chain fatty acid is gamma-linolenic acid (GLA). It formed a level of 1.07-1.27 % in the snails. It is found in evening primrose, borage and black currant oils. The body makes GLA out of omega-6 linoleic acid and uses it in the production of substances called prostaglandins, localized tissue hormones that regulate many processes at the cellular level. Eicosadienoic acid [C20:2 *cis-11*, 14 or 20:2 *(n*-6) all-*cis*-11, 14-eicosadienoic acid] or homo-gamma-linoleic acid is an uncommon naturally occurring PUFA. It is not enriched in any particular tissue, it is rare in all lipid classes. Dietary sources include herring and menhaden oils, cattle liver, swine brain lipid, shark oil (Yagaloff *et al.*, 1995). Homo-γ-LA had levels of 8.36-16.7 % in the total fatty acids of the snails, being the highest concentrated among the total PUFA FAs or 32.8-43.2 % of the PUFA. The FA inhibits the binding of [^{3}H]-ITB$_4$ to pig neutrophil membrane with a Ki of 3μm.

The levels of C18:2 *cis* -9, *trans*-11 ranged from 7.37-9.77 % as seen in Table 3. These levels were more than their corresponding LA (7.30-9.51 %, also Table 3). In Table 2 vaccenic acid levels ranged from 3.17-3.94 %. Conjugated linoleic acids make up a group of polyunsaturated FAs found in meat and milk from ruminant animals and exist as a general mixture of conjugated isomers of LA. Of the many isomers

identified, the *cis*-9, *trans* -11 CLA isomer (also referred to as rumenic acid or RA) accounts for up to 80-90 % of the total CLA in ruminant products (Nuernberg *et al*., 2002). Naturally occurring CLAs originate from two sources: bacterial isomerization and/or biohydrogenation of trans-fatty acids in the adipose tissue and mammary glands (Griinari *et al*., 2000). Microbial biohydrogenation of LA and aLA by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens* is highly depend on rumen pH (Pariza *et al*., 2000). Grain consumption decreases rumen pH, reducing *B. fibrisolvens* activity, conversely grass-based diets provide for a more favourable rumen environment for subsequent bacterial systhesis (Bessa *et al*., 2000). Rumen pH may help to explain the apparent differences in CLA content between grain and grass-finished meat products. *De novo* synthesis of CLA from 11*t*-*C*18:1 TVA has been documented in rodents, dairy cows and humans. Studies suggest a linear increase in CLA systhesis as the TVA content of the diet increased in human subjects (Turpeinen *et al*., 2002). The rate of conversion of TVA to CLA has been estimated to range from 5 to 12 % in rodents to 19 to 30 % in humans (Turpeinen *et al*., 2002). True dietary intake of CLA should therefore consider native 9*c*11*t*-C18:2 (actual CLA) as well as the 11*t* - C18:1 (potential CLA) content of foods (Adlof *et al*., 2000).

Over the past two decades numerous studies have shown significant health benefits attributable to the actions of CLA, as demonstrated by experimental animal models, including actions to reduce carcinogenesis, atherosclerosis, and onset of diabetes (Kritchevsky *et al*., 2000). Conjugated LA has also been reported to modulate body composition by reducing the accumulation of adipose tissue in a variety of species including mice, rats, pigs, and now humans (Smedman and Vessby, 2001). Optimal dietary intake remains to be established for CLA. It has been hypothesized that 95 mg CLA/day is enough to show positive effects in the reduction of breast cancer in women utilizing epidemiological data linking increased milk consumption with reduced breast cancer (Knekt *et al*., 1996). Ha *et al*. (1989) published a much more conservative estimate stating that 3 g/day CLA is required to promote human health benefits. Ritzenthaler *et al*. (2001) estimated CLA intakes of 620 mg/day for men and 441 mg/day for women are necessary for cancer prevention. Obviously, all these values represent rough estimates and are mainly based on extrapolated animal data. What is clear is that we as a population do not consume enough CLA in our diets to have a significant impact on cancer prevention or suppression. Reports indicate that Americans consume between 150-200 mg/day, Germans consume slightly more between 300-400 mg/day (Ritzenthaler *et al*., 2001), and the Australians seen to be closer to the optimum concentration at 500-1000 mg/day according to Parodi (1994).

The relative values of PUFA in all the samples made them important in diet. The eicosanoids help regulate blood clot formation, blood pressure, blood lipid (including cholesterol) concentration, the immune response, the inflammation response to injury and infection and many other body functions (Whitney *et al*., 1994). A deficiency of *n-6* fatty acids in the diet leads to skin lesions. A deficiency of *n-3* fatty acids leads to subtle neurological and visual problems. Deficiencies in PUFA produce growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in those fatty acids (Tapiero *et al*., 2002). The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and polyunsaturated fats (Honatra, 1974). The present PUFA/SFA varied between 0.512-1.03 which were averagely normal. The *n*-6 and *n*-3 FAs have critical roles in the membrane structure (Kinsella, 1990) and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the *n*-6 and *n*-3 FAs in the diet can be of considerable importance (WHO/FAO, 1994). The ratio of *n-6* to *n-3* or specifically LA to aLA in the diet should be between 5:1 and 10:1 (WHO/FAO, 1994) or 4-10 g of *n-6* FAs to 1.0g of *n-3* FAs (Canadian Government Publishing Center, 1990). As LA is almost always present in foods, it tends to be relatively more abundant in animal tissues. This is supported in the present report as follows: C18:2 (*n*-6) ranged as 7.30-9.51 % whereas C18:3(*n*-3) ranged as 1.37-1.65 %. In turn, these FAs are the biosynthetic precursors in animal systems of C20 and C22 PUFAs, with 3-6 double bonds, via sequential desaturation and chain-elongation steps (desaturases in animal tissues can only insert a double bond on the carboxyl side of an existing double bond) (Berg *et al*., 2007). Whilst it would be easy for the body to synthesize arachidonic acid [20:4(*n*-6)] from [18:2(*n*-6)], it may be difficult to synthesize the *n*-3 PUFA series especially eicosapentaenoic acid [20:5(*n*-3) or EPA] because of the low level of C18:3(*n*-3) and so the diet must be enhanced in this PUFA. However, the 2*n*-6/3*n*-3 fell within the above ratio as 5.3:1, 5.3:1 and 6.2:1.

Literature results for MUFA were: beef fat (48 %), lamb fat (39 %), pork fat (41 %), chicken, meat and skin (42 %), duck, meat and skin (54 %) and calf, liver (54 %); their corresponding PUFA were: beef fat (4 %), lamb fat (5 %), pork fat (15 %), chicken, meat and skin

(19 %), duck, meat and skin (12 %), calf, liver (26 %) (Bender, 1992). All the snail MUFA levels were lower than the literature values shown above as the MUFA snail levels were 23.8-24.8 %. On the other hand all the PUFA levels in the snails were higher than the literature PUFA levels shown above since snails had levels of 25.5-38.7 %. The C18:2 levels from the literature were (in %): rabbit, lean (13.5), brain, sheep (0.4), liver: ox (7.4), sheep (5.0), pig (14.7), calf (15.0) (Paul and Southgate, 1978) which were highly comparable with the snail results at 7.30-9.51 %. From literature for C18:3, we had (in %): rabbit, lean (0.7), brain, sheep (-), liver: ox (2.5), sheep (3.8), pig (0.5), calf (1.4) (Paul and Southgate, 1978); these results were highly comparable to the snail C18:3 results of 1.37-1.65%.

The statistical analysis of the results in Tables 2 and 3 as summarised in Table 4 showed that all the results in the row columns were not significantly different at $\alpha = 0.05$. On the other hand all the vertical column results were significantly different among themselves at $\alpha = 0.05$. It should be noted particularly for cases where there are more than two categories or groups that X^2 cannot indicate or specify where the significant difference lies, a situation similar to that found in ANOVA. However, post hoc tests that provide solution to the problem when encountered in ANOVA cannot be applied to Chi Square test. In case of X^2 – test, the category that contributes the highest proportion is declared as one that differs significantly from others (Oloyo, 2001). This meant that the SFA was significantly different from other members in each vertical column since it contributed the highest proportion.

b) Phospholipids

Table 5 shows the level of various phospholipids in the samples. Phospholipids are not essential nutrients: they are just another lipid and, as such, contribute 9 kcalories per gram of energy. Minor contributors to the phospholipids level were phosphatidylethanolamine (PE), phosphatidylserine (Ptd-L-Ser or PS), lysophosphatidylcholine and phosphatidylinositol (PI), each of them contributed less than 1.0 mg/100 g in each of the snail samples. The total phospholipids level ranged from 1.55-2.88 mg/100 g showing the snails to be low in phospholipid content. The only phospholipid with values closer to 1.0 mg/100 g was lecithin (phosphatidylcholine) with values range of 0.958-1.72 mg/100 g. Lecithin is usually the most abundant phospholipid in animals and plants, often amounting to almost 50 % of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin values in these results with percentage values ranging from 57.5-61.8 %. Phosphatigylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Phosphatidylcholines are such a major component of lecithin that in some contexts the terms are sometimes used as synonyms. However, lecithin extract consists of a mixture of phosphatidylcholine and other compounds. It is also used along with sodium taurocholate for stimulating fed- and fasted-state biorelevant media in dissolution studies of highly-lipophilic drugs. Phosphatidylcholine is more commonly found in the exoplasmic or outer leaflet of a cell membrane. It is thought to be transported between membranes within the cell by phosphatidylcholine transfer protein (PCTP) (Wirtz, 1991). Phosphatidylcholine also plays a role in membrane-mediated cell signalling and PCTP activation of other enzymes (Kanno *et al.*, 2007). At birth and throughout infancy, phosphatidylcholine concentrations are high (as high as 90 % of the cell membrane), but it is slowly depleted throughout the course of life, and may drop to as low as 10 % of the cellular membrane in the elderly. As is such, some researchers in the fields of health and nutrition have begun to recommend daily supplementation of phosphatidylcholine as a way of slowing down senescence (Mei-Chu, 2001) and improving brain functioning and memory capacity (Chung *et al.,* 1995). In addition to the increased caloric burden of a diet rich in fats like phosphatidylcholine, a recent report has linked the microbial catabolites of phosphatidylcholine with increased atherosclerosis through the production of choline, trimethylamine oxide and betaine (Wang *et al.*, 2011). The present snail samples were all low in both total fat and phosphatidylcholine. In Table 6, the X^2 values at both the row column and vertical column were not significantly different at $\alpha = 0.05$.

c) Sterols

The sterol results in Table 7 showed the values to be low at 37.1-45.1 mg/100 g and close in the samples with CV % of 9.86. Only cholesterol was greater than 0.00 mg/100 g in the samples. Cholesterol is a high-molecular-weight alcohol that is manufactured in the liver and in most human cells. Like saturated fats, the cholesterol we make and consume plays many vital roles. Along with saturated fat, cholesterol in the cell membrane gives our cells necessary stiffness and stability. This is why serum cholesterol levels may go down temporarily when we replace saturated fats with polyunsaturated oils in the diet (Jones, 1997). Cholesterol acts as a precursor to vital corticosteroids, hormones that help us deal with stress and protect the body against heart disease and cancer; and to the sex hormones like androgen, testosterone, estrogen and progesterone. Cholesterol is a precursor to vitamin D, a

very important fat-soluble vitamin needed for healthy bones and nervous system, proper growth, mineral metabolism, muscle tone, insulin production, reproduction and immune system function. The bile salts are made from cholesterol. Bile is vital for digestion and assimilation of fats in the diet. Recent research shows that cholesterol acts as an antioxidant (Cranton and Frackelton, 1984). This is the likely explanation for the fact that cholesterol levels go up with age. As an antioxidant, cholesterol protects us against free radical damage that leads to heart disease and cancer. Cholesterol is needed for proper function of serotonin receptors in the brain (Engelberg, 1992). Serotonin is in the body's natural "feel-good" chemical, low cholesterol levels have been linked to aggressive and violent behaviour, depression and suicidal tendencies. Mother's milk is especially rich in cholesterol and contains a special enzyme that helps the baby utilise the nutrient. Babies and children need cholesterol-rich foods throughout their growing years to ensure proper development of the brain and nervous system. Dietary cholesterol plays an important role in maintaining the health of the intestinal wall (Alfin-Slater and Aftergood, 1980). This is why low-cholesterol vegetarian diets can lead to leaky gut syndrome and other intestinal disorders.

Cholesterol levels in literature from many animal protein sources were much higher than the snail results. Values in mg/100 g were: fish (50-60), egg yolk (1260), meat and poultry (60-120), brain (2000-3000), liver (300-350) (Bender, 1992); others were rabbit, lean (71), brain, sheep (2200), liver: ox (270), sheep (430), pig (260) and calf (370) (Paul and Southgate, 1978). However the snail cholesterol levels were higher than in the fish (mg/100 g): 6.86 (skin) and 0.303 (muscle) of Tongue sole fish (Adeyeye *et al.*, 2011); 31.6 (skin) and 4.35 (muscle) of *Oreochromis niloticus* (Adeyeye, 2011). Most authorities, but not all, recommend a reduction in dietary cholesterol to around 300 mg or less per day (Bender, 1992); all the snail results were much lower than 300 mg.

d) Quality assurance

The correlation determined for all the standards: fatty acids, phospholipids and sterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (sterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results, thus attesting to the quality assurance of the determinations.

V. Conclusion

The findings of this study showed that the samples demonstrated the lipid concentration of ruminants with slight unequal distribution of all parameters determined. The samples were low in total fats, low concentration of cholesterol and phospholipids. All the samples had unsaturated acids as the predominant fatty acids with good percentage levels of C18:*1 cis-6*, C18:1 *cis*-9, C18:1 *trans*-6, C18:1 *trans*-11, C18:2 *cis* 9, 12, C20: 2 *cis*-11, 14 and *C*18:2 *cis*-9, *trans* 11. Significant differences occurred in the fatty acid levels. Quality assurances of the determinations were highly satisfactory.

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Table 1 : Crude fat and total energy levels of three different types of land snails in Nigeria

Parameter	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Crude fat (g/100 g)	2.38	2.35	2.22	2.32	0.09	3.67
Total energy (kJ/100 g)*	88.1	87.0	82.1	85.7	3.19	3.73

**Crude fat x 37 kJ; SD = standard deviation; CV % = coefficient of variation.*

Table 2 : Saturated and monounsaturated fatty acid composition of three different types of land snails in Nigeria (% total fatty acid)

Fatty acid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Acetic acid (C2:0)	Nd	Nd	Nd	-	-	-
Propionic acid (C3:0)	Nd	Nd	Nd	-	-	-
Botanic acid (C4:0)	Nd	Nd	1.09	-	-	-
Pentanoic acid (C5:0)	0.00	0.00	0.00	0.00	0.00	0.00
Hexanoic acid (6:0)	Nd	Nd	Nd	-	-	-
Octanoic acid (C8:0)	Nd	Nd	Nd	-	-	-
Decanoic acid (C10:0)	0.143	0.069	0.152	0.122	0.046	37.7
Lauric acid (C12:0)	Nd	0.179	Nd	-	-	-
Myristic acid (14:0)	0.240	0.116	0.256	0.204	0.077	37.6
Palmitic acid (C16:0)	16.6	13.6	19.4	16.5	2.90	17.5
Stearic acid (C18:0)	25.8	23.4	28.7	26.0	2.65	10.2

Arachidic acid (C20:0)	0.00	0.00	0.00	0.00	0.00	0.00
Behenic acid (C22:0)	0.215	0.104	0.229	0.183	0.068	37.5
Lignoceric acid (C24:0)	0.00	0.00	0.00	0.00	0.00	0.00
Total SFA	**43.0**	**37.5**	**49.8**	**43.4**	**6.16**	**14.2**
Myristoleic acid(C14:1*cis*-9)	0.445	0.256	0.363	0.355	0.095	26.7
Palmitoleic acid (C16:1*cis*-9)	0.086	0.042	0.092	0.073	0.027	37.2
Petroselinic acid(C18:1*cis*-6)	3.41	3.33	2.43	3.06	0.544	17.8
Oleic acid (C18:1 *cis*-9)	2.53	2.41	6.11	3.68	2.10	57.1
Gondoic acid (C20:1 *cis*-11)	0.143	0.069	0.152	0.121	0.046	37.5
Erucic acid (C22:1 *cis*-13)	0.00	0.00	0.00	0.00	0.00	0.00
Nervonic acid (C24:1*cis*-15)	0.00	0.00	0.00	0.00	0.00	0.00
MUFA (*cis*)	**6.61**	**6.11**	**9.15**	**7.29**	**1.63**	**22.4**
trans-Petroselinic (C18:1*trans*-6)	8.44	8.37	6.35	7.72	1.19	15.4
Elaidic acid (C18:1 *trans*-9)	5.08	5.35	6.07	5.50	0.512	9.30
Vaccenic acid (C18:1*trans*-11)	3.83	3.94	3.17	3.65	0.416	11.4
MUFA (*trans*)	**17.4**	**17.7**	**15.6**	**16.9**	**1.14**	**6.72**
MUFA (totals)	**24.0**	**23.8**	**24.8**	**24.2**	**0.529**	**2.19**

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; Nd = not deleted; - = not determined.

Table 3 : PUFA *n*-6 and *n*-3 fatty acid composition of three different types of land snails in Nigeria (% total fatty acid)

Fatty acid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Linoleic acid (C18:2 *cis*-9, 12)	8.75	9.51	7.30	8.52	1.12	13.2
Gamma-linolenic acid (C18:3 *cis*-6,9,12)	1.27	1.21	1.07	1.18	0.103	8.67

Eicosadienoic acid						
(C20:2 *cis*-11,14)	12.6	16.7	8.36	12.6	4.17	33.2
Dihomo-γ-linolenic acid						
(C20:3 *cis*-8, 11, 14)	0.00	0.00	0.00	0.00	0.00	0.00
Arachidonic acid (AA)						
(C20:4 *cis* 5, 8, 11, 14)	0.00	0.00	0.00	0.00	0.00	0.00
Docosadienoic acid						
(C22:2*cis*-13,16)	0.00	0.00	0.00	0.00	0.00	0.00
***n*-6 PUFA (*cis*)**	**22.6**	**27.4**	**16.7**	**22.2**	**5.36**	**24.1**
Rumenic acid						
(C18:2 *cis*-9, *trans*-11)	8.84	9.79	7.37	8.67	1.22	14.1
***n*-6 PUFA (totals)**	**31.4**	**37.2**	**24.1**	**30.9**	**6.56**	**21.2**
Alpha-linolenic acid (ALA)						
(C18:3 *cis*-9, 12, 15)	1.65	1.54	1.37	1.52	0.141	9.28
Eicosatrienoic acid (ETE)						
(C20:3 *cis-11, 14, 17*)	0.00	0.00	0.00	0.00	0.00	0.00
Cervonic acid (DHA)						
(C22:6 *cis*-4,7,10,13,16,19)	0.00	0.00	0.00	0.00	0.00	0.00
***n*-6 + *n*-3 (PUFA)**	**33.1**	**38.7**	**25.5**	**32.4**	**6.63**	**20.4**
Totals (SFA+MUFA+PUFA)	100	100	100	100	0.00	0.00
Totals (MUFA +PUFA)	**57.1**	**62.5**	**50.3**	**56.6**	**6.11**	**10.8**
PUFA/SFA	0.770	1.03	0.512	0.771	0.259	33.6
MUFA/SFA	0.558	0.635	0.498	0.564	0.069	12.2
2*n*-6/3*n*-3	5.30	6.18	5.33	5.60	0.500	8.92
Ratio	1:1	1:1	1:1	-	-	-

PUFA = unsaturated fatty acid.

Table 4 : Statistical analysis of the results from Table 2 and 3

Fatty acid	*A. marginata*	*Archatina archatina*	*Limicolaria* sp	X^2	Remark
SFA	43.0	37.5	49.8	1.77	NS
MUFA	24.0	23.8	24.8	0.021	NS
DUFA	30.2	36.0	23.0	2.85	NS
TUFA	2.92	2.75	2.44	0.045	NS
X^2	33.5	30.9	45.1	-	-
Remark	*	*	*	-	-

*X^2 = chi-square; NS = not significant at $\alpha_{=0.05}$ and critical value of 5.99; * = significant at $\alpha_{=0.05}$ and critical value of 7.82; DUFA = diunsaturated fatty acid; TUFA = triunsaturated fatty acid.*

Table 5 : Phospholipids level (mg/100 g) of three different types of land snails in Nigeria

Phospholipid	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Cephalin (PE)	0.450 (15.6)	0.383 (13.4)	0.226 (14.6)	0.353	0.115	32.6
Lecithin	1.72 (59.7)	1.64 (57.5)	0.958 (61.8)	1.44	0.419	29.1
Ptd-L-Ser (PS)	0.244 (8.47)	0.35 (12.3)	0.186 (12.0)	0.260	0.084	32.2
Lysophosphati-dylcholine	0.209 (7.26)	0.214 (7.51)	0.088 (5.68)	0.170	0.071	41.9
PtdIns (PI)	0.263 (9.13)	0.261 (9.16)	0.096 (6.19)	0.207	0.096	46.4
Totals	2.88	2.85	1.55	2.43	0.759	31.3

PE = phosphatidylethanolamine; Lecithin = phophatidylcholine; PS = phosphatidylserine; PI = phosphatidylinositol; values in parentheses are in percentages. 14

Table 6 : Statistical analysis of the results from Table 5

Phospholipid	*A. marginata*	*Archatina archatina*	*Limicolaria* sp	X^2	Remark
PE	0.45	0.383	0.226	0.075	NS
Lecithin	1.72	1.64	0.958	0.243	NS
PS	0.244	0.351	0.186	0.054	NS
Lysophospha-tidylcholine	0.209	0.214	0.088	0.059	NS
PI	0.263	0.261	0.096	0.088	NS
X^2	2.88	2.56	1.73	-	-
Remark	NS	NS	NS	-	-

NS = not significant at $\alpha_{=0.05}$ (critical value = 5.99) on the row and critical value 9.49 at the column.

Table 7 : Sterol level (mg/100 g) of three different types of land snails in Nigeria

Sterol	*A marginata*	*Archatina archatina*	*Limicolaria* sp	Mean	SD	CV%
Cholesterol[+]	42.7	45.1	37.1	41.6	4.11	9.86

[+] X^2 value was 0.805 and not significant at $\alpha_{=0.05}$ and critical value of 5.99.