DETERMINATION OF SOME HEAVY METALS AND INVESTIGATION OF NUTRITIONAL COMPOSITION IN THREE SPECIES OF SNAILS (*ARCHATINAFULICA, ARCHACHATINA MARGINATA* AND *LIMICOLARIAFLAMMEA)*

BY

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M.SC/SCIE/12633/2011-2012

DEPARTMENT OF CHEMISTRY
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.

OCTOBER, 2015.

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA,
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DEPARTMENT OF CHEMISTRY
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OCTOBER, 2015.

Declaration

I declare that the work in this dissertation entitled**"Determination of some heavy metals and investigation of Nutritional Composition in three Species of snails. "*Archatinafulica, Archachatina Marginata* and *Limicolariaflammea*"**was carried out by me in the Department of Chemistry. The information derived from the literatures has been duly acknowledged in the text and in the list of references provided. No part of this dissertation was previously presented for another degree in this or any other institution.

BeatriceKADANGA _____ _____

Signature Date

Certification

This dissertation entitled **"Determination of some heavy metals and investigation of Nutritional Composition in three Species of snails**(***Archatinafulica,Archachatina Marginata*** **and** ***Limicolaria flammea)*** by Beatrice Kadanga meets the regulations governing the award of Master Degree in Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to Late Mrs. B. G. Kadanga, Mrs. J. H. Kadanga and to my Daughter Miriah Musa.

Acknowledgement

My profound gratitude goes to my supervisors Prof. C. E. Gimba and Prof. V. O. Ajibola who took their time to supervise this research work.

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Finally, I thank the almighty God and return all glory to him.

Abstract

Species of snails *Archachatina marginata,Archatinafulica and Limicolaria flammea*, and *Archachatina marginata* were assessed for proximate analysis aimed at establishing their nutritive values. The heavy metals levels of the flesh was also investigated. Analysis of the flesh revealed that the composition of crude protein varied from 34.90± 0.23 to 36. 75 ± 0.22% in all species, Ash content, moisture content, crude lipid, and carbohydrateranged from 6.15± 0.99 to 16.06 ± 0.75%, 9.41± 0.33 to 27. 89± 0.52%, 7.29± 0.63 to 9.99± 0.15%, 14.71± 0.17 to 38.18± 0.66% respectively. Crude fibre was not detected in any of the species.The concentration of zinc, copper, cadmium and lead in the flesh of the snails were in the range of 7.58± 0.03 to 13.10± 0.03 mg/kg, 0.99± 0.01 mg/kg to 3.32±0.01 mg/kg, 0.09± 0.00 to 0.50± 0.00 mg/kg, and 0.90± 0.00 to 2.90±0.00 mg/kg respectively.These values were low and within the WHO permissible limit. Amino acid profile indicates the presence of lysine, Alanine, Histidine, valine, leucine and Phenylalanine Arginine, Aspartic acid, threonine serine, Glutamic acid, proline, Glycine, Cystine, Isoleucine, Leucine and Tyrosine in the snail species. Lysine concentration varied from 15.67-16.00mg/g in all species. Histidine was 2.50.73mg/g, Arginine ranged from 16.11-16.85. Aspartic acid ranged from 9.66 – 10.54mg/g, Threonine concentration ranged from 8.34-9.01mg/g, serine (4.55 – 4.99 mg/g), Glutamic acid (11.64 – 11.94mg/g), proline (5.80–6.26mg/g), Glycine (4.01-4.86 mg/g), Alanine (2.98 – 3.31 mg/g), cystine (0.55-0.69mg/g), valine (7.9 – 8.21mg/g), methionine (1.93 -1.98mg/g), Isoeucine (8.91 – 9.00mg/g), leucine (9.51 – 9.98mg/g), Tyrosine (4.63 – 4.80 mg/g), phenylalanine (6.66 – 7.13 mg/g). Ammonia and Norleucine were not detected in all the snail samples. The result shows that snailcould complement the required trace minor elements needed for proper growth and development in human and hence recommended for regular consumption.

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Abbreviations

AF	-	*Archatina fulica*
AM	-	*Archachatina marginata*
LF	-	*Limicolaria flammea*
C	-	Control sample
AOAC	-	Association of Official Analytical Chemist
EPA	-	Environment Protection Authority
DWAF	-	Department of water Affair and Forestry
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Snail is a common name that is applied most often to land snails, terrestrial pulmonate, gastropod mollusce. Snail is also applied to most of the members of the molluscan class gastropoda that have a coiled shell large enough for theanimal to retract completely into. “Snail” is used in this most general sense; it includes not just land snails but also thousands of species of sea snails and fresh water snails. Snail-like animals that naturally lacka shell, or have only an internal shell, are usually called slugs, and land snails that have only a very small shell that they cannot retract into are often called semislugs.

Snails are one of the earliest known types of animals in the world. They are able to adapt to a variety of living condition and they do not require large amounts of food. They have been able to continually evolve to survive the condition around them which many researchers find to be very fascinating. All snails are classified as mollusks because of the hard shell that protects their bodies; snails are found in many locations and have a very diverse type of habitat where they can be found. As a snail moves it leaves behind a trail of slims, this allows it to easily move across any type of terrain without injuring itsbody. They are not able to hear at all. So they rely on their sense of touch to interact with each other. They use their sense of smell to help them find food. You will find that snails are the most active at night and may come out during the early morning hours as well(Frederick, 2010).

Snails have prominent tentacles on which, in many species, the eyes are often located, many snails are as small as 0.1cm (0.04inch) long; others, such as conches and

the African land snail, are as long as 20cm (8inch). The spiral shell into which the snail withdraws serves mainly as protection against predators and desiccation. Land snails are particularly well adapted to changes in moisture and some desert species are able to remain sealed within their thick shells for two or more years. Land snail species of more moist habitats usually have thinner shells. Slugs, which live in very moist places and are often considered snails, have only vestigial shells.

Snails can be found in a very wide range of environments, including ditches, deserts, and the abyssal depths of the sea. Although land snails may be more familiar to people, marine snails constitute the majority of snails' species and have much diversity**.** Numerous kinds of snail can also be found in fresh water.

Most snails have thousands of microscopic tooth-like structures located on a ribbon-like tongue called a radula used to cut food into small pieces. Many snails are Herbivorous, eating plants or rasping algae from surfaces with their radula. However, a few land species and many marine species are omnivores, predatory or carnivores. The life span for snails depends on their habitat and the species. Some of them only live for about 5 years however; others in the wild are believed to live at least 25 years old. The life span of snails is decreasing due to humans destroying their habitat and due to pollution.

1.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Mollusca
Class:	Gastropoda

(Fredericks, 2010)

1.2 Common Names Of Snails

English	-	Snail
Hausa	-	Dodon kodi
Igbo	-	Ejule
Yoruba	-	Igbin



Figure 1: A typical land snail

1.3 Economic Importance Of Snail

The economic and medicinal benefits from snails are immense. These run from their foot tissue to shells (Wosu, 2003). They serve as valuable sources of nutrition to human and animals with high levels of protein, iron, lysine, leucine, arginine, calcium and phosphorus, relatively low amount of sodium, fat and cholesterol compared to poultry and other livestock[Thompson, (1996); Wosu. (2003)].Many common health problems can be prevented or alleviated with a healthy diet (Clinical Nutrition Board, 2011). Our bodies need the correct balance of nutrients in food we take to function optimally.Snails are cheap to rear at subsistent and commercial levels with high returns on low input.

Traditionally, snail is used in the preparation of concoctions for various cases such as reduction of labour pains, blood loss during delivery and in the cure of small pox (Akinnusi, 1997). It is also implicated in the treatment of anaemia, hyperension, high blood pressure and other fat related ailments (Wosu,2003).

Umoh and Bassir (1977) and Mba (1980) highlighted a good number of lesser known animals foods such as clam, periwinkle and land snails.The consumption of these food were limited to a small population owing to lack of adequate information about their nutritional potential.

It has been revealed by Imevbore and Ademosun (1988) that land snails meat contains as high as 88.3 of protein. Adeyeye (1996) reported 20.6%, 14.52% and 17.51% of protein in *Archachatina marginata, Archatina* species and *limicolaria spp* respectively. *Lymnaen stangials* (Pond snail) popularly called "Igbin Oluweri" by Yoruba tribe of western Nigeria, is a species of snails and belongs to the class gastropoda.It is used as food by the "Oluweri", (Pond or river worshippers) and is available all year round in ponds(Yusuf,2004). The economy importance of snail can be classified as follows:

i. **Agriculture:**The practice ofrearing snails for food is known as heliciculture. The snails are kept in a dark place in a wired cage with dry straw or dry wood-coppiced wine-grape vines. During the rainy period the snails come out of hibernation and release most of their mucus onto the dry wood/straw. The species use for these purpose are known as agricultural and garden pest. They are edible delicacy, and occasionally a household pet.

ii. **Cosmetic:** recently, *Halix aspersa* has gained some popularity as the chief ingredient in skin creams and gels (cream/gel de carecol) sold within the Latino community in the USA. These creams are promoted as being suitable for use on wrinkles, scars, dry skin and acne(Andes, 2006).A screen of the secretions that *Halix aspersa*snail produces under stresspossesses pharmacological properties(skin-regenerative properties). The secretions contain antioxidant, superoxide, dismutase and glutathione S-transferase (GSTS). The secretions stimulate fibroblast proliferation, stimulation of extracellular matrix assembly and the regulation of metalloproteinaseactivities.

iii. **Cultural Depiction:** Due to its slowness, the snail has traditionally been seen as a symbol of laziness. In Christian culture, it has been used as a symbol of the deadly sin of sloth. Psalm 58:8 uses snail slime as a metaphorical punishment. The Greekpoet Hesiod wrote that snails signified the time of harvest by climbing the stalks, while the Aztec moon god tecciztecatl bore a snail shell on his back. This symbolized rebirth; the snail penchant forappearing and disappearing was analogized with the moon (Andes, 2006).

iv. **Snail as Food:** several species of land snails provide and easily harvested source of protein to many people in poor communities around the world. Many land snails are valuable because they can feed on a wide range of agricultural wastes, such as shed leaves in banana plantations. In some countries, giant African land snails are produced commercially for food. Land snails, freshwater snails, and sea snails are all eaten in a number of countries. (principally spam, Philippines, morocco, Nigeria, Algeria, Cameroon, France, sicily, Portugal, Greece, Belgium, Vietnam, Laos,

Cambodia, Cyprus, Ghana, malta, teraiofnepal, southwestern China, northeast India states such as Manipur, Tripura and parts of the USA).

1.4 Heavy Metals

Heavy metals are elements with atomic weight between 63 and 201 and specific gravity above 5.0. They are metals with high density and toxic or poisonous at low concentration (Lenntech, 1998).

Heavymetals occur naturally in the eco-system with large variation in concentrations. They cannot be degraded or destroyed to a small extent, and hence enter our bodies via food chain, drinking water and air. High concentrations of heavymetal can lead to poisoning. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damages to blood composition, lungs, kidney sliver and other vital organs. Long – term exposure may result in slowly progressing muscular and neurological degeneration process that mimic Al$_Z$heimer's diseases, parkion's disease, muscular dystrophy and uncommon and repeated long – term contact with some metals or their compound may a case cancer (John,2002).

Copper and Zinc have essential physiological and biochemical functions in plants and animals (Schlosser*et al.*, 2007). However, excessive levels can be damaging to environmental health (Murariu*et al.*,2010). Cd and Pb have no known vital or beneficial effect on organisms (Nordbery *et al.*, 2002), except for diatoms, where a Cd – based enzyme play an essential role in role in regulating atmospheric carbon Lane *et al.*, (2005).

Laskowski and Hopkin (1996) showed that land snails can concentrate high levels of Cu, Zn Cd and Pb in their soft tissue without revealing any major metabolic disorder. These heavy metals were found to accumulate into the snail shell, often resulting in

alterations of shell geometry or size. In polluted soils Cu, Zn, Cd, and Pb are taken by vegetation, and subsequently they can be transferred along food chain to herbivorous organism. Cu, Zn, Cd, and Pb accumulate in dead plant and animal materials, and thereafter, they are ingested by organisms that feed on decaying organic matter (detritivore organism). Because snails act on tropic level, both as herbivorous and deritivore organism, they can be involved in heavy metals biomagnifications along the food chain Notten *et al*., (2005).

Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. Cadmium is bio-persistent and once absorbed by an organism, remains resident for many yearsin humans. Long-term exposure is associated with renal dysfunction, high exposure can lead to obstructive lung disease and has been linked to lung cancer.Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. The metal can be linked to increased blood pressure and effects on the myocardium in animals(Lenntech, 1998).

Copper is an essential substance in human life, but in high doses, it can cause anemia, liver and kidney damage. People with Wilson's disease areat greater risk for health from over exposure to copper. Copper normally occurs indrinking water from copper pipes, as well as from additive, designed to control algal growth (Lenntech, 1998).

Human'sexposure to lead can result in a wide range ofbiological effect depending on level and duration ofexposure. High levels ofexposure may result to toxic biochemical effects in humans whichinturn cause problems in the syndissertation of hemoglobin, effects on kidneys, gastrointestinal tract, joints, reproductive system and acute or aleuronic damage to the nervous system (Afal and Wiener, 2014). Lead poisoning, which

is so severe as to cause evident illness, is now very rare indeed. At intermediate concentrations, there is persuasive evidence that lead can have small, subtle sub-clinical effects, particularly on neuropsychological developments in children. Some studies suggest that there may be a loss of up to 21Q points for a dry in blood lead levels from10to 20mg/dl in young children (Afal and Wiener, 2014).Average daily lead intake for adults intheUK is estimated at 1.6 mg from air, 20mg from drinking water and 28mg from food (WHO, 1993).In adult population the major exposure pathway is from food and water. Food, air, water and dust/soil are the major potential exposure path ways for infants and young children (Afal and wiener, 2014).

Zinc is needed by human and other animals in small amounts, but in larger amount, it can cause damage to the nervous system, fatigue and irritability(Afal and Wiener, 2014). Zinc accumulates in living tissue, causing high zinc content in fish and other organisms and causing greater health problem in human over a lifetime of over exposure. These health problems include hair and finger nail loss, damage to kidney and liver tissue, damage to circulatory tissue and more severe damage to the nervous system(Lenntech, 1998).

1.5. Justification Of The Study

A major task facing the world today is that of providing sufficient food of adequate quality for the rapidly increasing human population. The food deficit situation is more intense with protein deficiency when compared to the availability of carbohydrate and the micro elements.Adesehinwa and Ogunmodede (1995) reported that humans have used snails for food for many generations.Despite this, most of the scientific work done; on snails have been from the point of view of animal's parasitology where snails act as

intermediate host of pathogenic nematodes(Wosu, 2003). Due to the increased in the demand and consumption of snails, it has become necessary to know the level of heavy metals concentrations present in snail in order to ascertain their safety for consumption.It is also imperative to investigate the amino acid profile to create awareness to the general public on their nutritional status.

1.7 Aim Of The Study

The aim of this research is to ascertain the concentration levels of some heavy metals in some species of snail and investigate their nutritional values.

1.8 Objective Of The Study

i. To determine the concentrations of copper, cadmium, zinc and lead in *Archatina fulica, Limicolaria flammea* and *Archachatina marginata* snails.

ii. To determine the nutritional values in the flesh of the snail species.

iii. To determine the amino acids present in the flesh of the snails species.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Studies on Bioaccumulation of Heavy Metals in Snails

Ezemonye and Ilechie (2010) did a study on bioaccumulation of heavy metal (Cu, Zn, Fe) in fresh water snail from Ikpoba River of Southern Nigeria. The study indicated the mean concentration of Cu, Zn and Fe in the snail to be in the range of 4.56 – 6.71, 35.35 - 77.09 and 1257.21 – 1762.59 (ugg^{-1}) respectively.The research shows that under natural conditions, snail is capable of bioaccumulating trace essential metals especially Fe and Zn up to 10 times higher than that found in the sediment.

Adedeji*et al* (2010) in a research work investigating heavy metals in snail and water samples from Alaro rivers in Oluyole industrial area of Ibadan, Southwestern Nigeria September, showed the estimated concentration and bio-concentration of lead, arsenic, chromium and cadmium. The concentration of the metals were 0.063± 0.009mgl^{-1} Chromium > Lead 0.006± 0.004mgl^{-1}> Cadmium 0.005± 0.003mgl^{-1} and > Arsenic 0.003± 0.001mgl^{-1}> Cadmium 0.005± 0.001mgl^{-1}> Arsenic 0.005± 0.003mgl^{-1}> Lead 0.004± 0.01mgl^{-1}. It was reported that there were no significant difference (p = 0.65, 0.45, 1, 0.53) in the levels of lead, arsenic, cadmium and chromium at between the water samples and the snail samples at 95% confidence limit. Heavy metals accumulated in indwelling snails are in the order Chromium > Lead > Arsenic > Cadmium. As long as water is polluted, it will not only affect water – dwelling organisms, but also terrestrial organism that feed on aquatic organisms. The animal which in turn, feed on these terrestrial animals will also be affected.

The effects of heavy metals on survival and growth of Biomphalaria Glabrata and interaction with Schistosome infection was investigated by Allah, and Thompson (1996). Pb at concentrations from 0.5-100μm, Cd from 0.075-0.25μm and Hg from 0.25-1μm significantly reduced growth and survival of normal snails.

The adsorption of heavy metals on modified snail (*Archachatina marginata*) shell was studied by Ikhuoria and Uyammadu(2001). The adsorption of cadmium and lead ions on modified powdered snail shell by equilibrium sorption studies at 29^{o}C. The high percent removal of the heavy metals shows that the snail shell could bioaccumulate heavy metals.

Topcuoglu(2002) investigated heavy metals levels in organism and sediments from Turkish coast of the black sea. The experimental organisms were macroalgae, sea snail, Mussel, and fishes. Heavy metal is analyzed were Cd, Co, Cr, Ni, Zn, Fe, Mn, Pb and Cu. The metal concentrations in macroalgae, sea snail, mussel and sediment samples were very high. The concentrations of Cd, Co, Cr, Zn, Fe, Mn, and Cu in sea snail, mussel and fish samples were correlated with that of the sediment.

A study conducted to determine the concentration of heavy metals (Zn, Pb and Cd) in Snail (*Archachatina marginata*) found in Ogun State was carried out by Sadiq (2003). 40 samples were collected from different Local Government Areas in the States and analysed with the aid of atomic absorption spactrophometer (AAS). The concentration of lead in the snail specimen analysed was found to be as low as 3.59 mg/kg in ijebu ode and as high as 9.03mg/kg in Imeko-mon Local Government Areas.The variation in metal content might not be unconnected with the rural and

cosmopolitan nature which determine the industrial and human activities that facilitate discharge of metals into the environment. Cadmium was not detected in all the specimens, which indicates that the level of cadmium introduced into the environment is significantly low. Also, the level of Zinc and lead determined in the wild snails were higher than in the control. Therefore, rearing of snails under intensive management should be encouraged

Iwegbue*et al*(2008)investigated heavy metal concentration in the African gaint snail *Archachatinamarginata* (Swainson) obtained from urine localities in southern Nigeria. The concentration of the metals ranged from 0.77 to 7.51 mg kg^{-1} Pb, 3.06 - 46.9 mg kg^{-1} Fe; 0.03-0.4-mg Kg^{-1} Zn; Cr Mn; 0.99-3.28 mg kg^{-1} Cd: 0.08-0.22 mg kg^{-1} Zn. Cr was below the limit of detection. Concentration of Pb, Fe, Ni, Cu, Co, Cd, and Zn in the snail tissue varied significantly (95% confidence limit) between the localities. The result indicates a heavy metal pollution of the habitats.

Wegwo and Wigwe (2006)investigate bio-accumulation of traced metals in the African giant land snail (*Archachatina marginata),* collected from six different states of southern Nigeria.The concentration of Zn, Ni, Pb and Cd were between 10.3-20.5, 3.20-10.4, 0.80-6.00 and 0.60-0.84 mg/kg respectively. The average concentration of trace metals recorded in the study remained below critical values.

Shuhaimi and Nadzifah(2012) reported that Cu was the most toxic metal to M. tuberculata, followed by Cd, Zn, Pb, Ni, Fe, Mn, and Al. Metals bio-concentrations in M. tuberculata increase with exposure to increasing concentrations and Cu has the highest accumulation (Concentration Factor) in the soft tissues of the snail.

Dragos*et al (*2012) study the bio-accumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain.Cu, Zn, Cd and Pb can pose serious threats to environmental health because they tend to bio-accumulate in terrestrial ecosystems. The investigation was done under field conditions. Cu, Zn, and Cd have magnified in the snail body, particularly in the hepatopancreas. In contrast, Pb decreased when going up into the food chain. Zn, Cd, and Pb correlated with that in soil. There were significant differences among sampling sites when compared with reference snails. The snail hepatopancreas accumulates high amounts of HMs, and therefore, this organ can function as a reliable biomarker for tracking HM bio-availability in soil, long-term exposure to HM via contaminated food might influence the variability of shell traits in snails' populations.

Gomot(2012) study the effects of heavy metals on snail development and investigate possible use of snails as bio-indicators of heavy metal pollution for the preservation of human health. They developed a test of toxicity based on the effects of a noxious and carcinogenic element, cadmium on the land snail *Helix aspersa aspersa* (H.a.a) of one mouth of age. Five concentrations (50 to 800 micrograms/g), were selected to estimate the concentrations causing's 50% inhibition of growth (EC 50) at 14days: 190 micrograms /g and at 28days: 180 mg/g. The ease of handling snails and the perfect control of their breeding are essential factors in carrying out reliable bioassays in toxicology and in ecotoxicology.

(Grara, *et al.*, 2012), study the effects of heavy metals on the snails *Helix aspersa* The results on metabolic level show that metal dust causes a significant increases in proteins with a significant reduction in the carbohydrates and lipids on the level of the

two studies bodies (digestive gland and the kidney).The exposure of *Helix aspersa* to metal dust induced a lipidic peroxidation with releases of maloudialdehyde (MDA) to the level of the studied bodies

Lefcort*et al (*2015) reported thatsnail from heavy-metal polluted environments have reduced sensitivity to carbon dioxide-induced acidity.They studied aquatic pulmonate snails (*physella Columbiana*) from both heavy-metal polluted watershed and snails from a reference watershed that has not experienced mining pollution. They predicted a simple negative additive effort of low pH. Snails from populations that acclimated or evolved in the presence of heavy metal mining pollution were more robust to acidic conditions than were snails from reference habitats. Snails from mining polluted sites seemed to be pre-adapted to a low pH environment. Their short-term survival in acidic conditions was better than snails from reference sites that lacked metal pollution.

Sharma and Uma(2009) in their study of effect of heavy metals (Cu and Pb) on the Gaint land snail, *Archachatinamarginta* (swainson), reported that exposure of the snails to sublethal concentrations of the metals resulted in a prevalence of hepatocellular Foci of Cellular Alteration (FCA) in the hepatopancreas of snails. Sharma and Uma, (2009) concluded that, the combination of these pathological end points with the monitoring of metal levels in body tissues of edible animals provides important tools for early detection of impending environmental problems and prevention of potential public health issues.

2.2 Nutritional Compositions of Snails

The snail is native to forests and areas of most vegetation in West African countries such as Cameroon, Cote d'voire, Ghana and Nigeria. Snail meat has feature in the diet of Asian countries, and of African people living in forested areas for thousands of years.

Akinsoyinu and Babalola(2009) investigated the proximate composition and mineral profile of snail's meat from different breeds of land snail in Nigeria. Four different breeds of snail, *Archachatina marginata* (AM), *Archatina archatina* (AA), *Archatina fulica* (AF) and *Limicolaria* species (LM) were analyzed. Proximate analysis of the snail flesh revealed that moisture was highest in LM (84.91%) and least in AM (73.6%). The crude protein contents of the breeds were significantly different from one another ($P<0.05$). The result of the mineral profile showed that AM recorded highest value (mg/100g) in calcium (126.40), iron (2.29) phosphorous (22.91), magnesium (25.01) and copper (1.03) while LM had the least values of 36.20, 0.72, 8.98, 5.28 and 0.29 (mg/100 g) respectively. There was no detection of lead and mercury in any of the samples. The result of the study showed that the African giant land snail, *Archacahtina marginata,* apart from having size advantage is the favoured breed based on its nutritional superiority.

Nutritional assessment of some Nigerian land and water snail species were arried out by Eneji *et al.,* (2008). The mean of crude protein ranged from 70.00 $\pm$ 0.03 to 84.43 $\pm$ 0.01%. Calcium values of 172.79 $\pm$ 0.01in *Limicolaria* spp to 182.70 $\pm$ 0.02mg / 100g in Nucalla lapillus were obtained.Phosphorus ranged from 51.49 $\pm$ 0.01 in *Archachatina*

marginata saturalis to 62.52 + 0.01 mg/100g in *Lanistes varicus* and iron content reanged from 6.9 + 0.06 in *Archachatina marginata* Ovum to 11.09 ± 0.01mg /100g in *Nucella lapillus* with low zinc content of 1.14 ± 0.01 in *Limicolaria* spp. to 1.31 ± 0.01mg/100g in *Nucella lapillus*.

Nutritional value and functional properties of pond snail (*Lymneea tagnsalis)*was reported by Yusuf and Oseni(2004). The moisture contents of the sample were 38.62g / 100 g and 30.06 g/ 100g dry matter (DM) for both the shell and the meat respectively. Crude protein for the meat was 25.60g / 100g DM while it was not detectable in the shells (ND). The crude fat and ash content for both the meat and the shell sample were 2.5g / 100g MD, 0.95g / 100 DM, 3.41 g / 100g DM and 52.41g / 100g DM respectively. Mineral element composition determination showed that *lymnaea stagnalis* meat is richer in sodium and potassium than the shell. While the shell is richer in calcium, nickel, magnesium, cadmium, iron, copper, and lead than the meat. Total oxalate was 420mg / 100g DM for the meat and 350mg / 100g DM for the shell, hydrocyanic acid was 180 mg / 200g DM for the meat and 121 mg/ 100g DM for the shell, while tannic acid was 520mg / 100g DM for he met 4800 mg / 100g DM for the shell.

The nutritional quality and organoleptic characteristics of the meat of the giant African land snail (*Archachatina marginata*) was compared to the meat of the conventional livestock species (Beef, rabbit and chicken) (Malik,*et al*., 2010).Each of the meat samples was processed using three different cooking methods (that is boiling, frying and barbecuing) and their organoleptic and sensory characteristics were evaluated (appearance and colour, juiciness, taste and general acceptability) using a 7 – point descriptive hedonic scale.Crude protein value was lowest in the chicken meat (18.3%),

slightly high in rabbit meat (20.0%) and beef (19.6%) and was highest in snail meat (20.5%). Showing that snail meat is a high quality protein food with the crude protein content higher than those of conventional livestock. But the meat of rabbit, chicken and beef were highly accepted for their appearance and colour, juiciness and taste when boiled, fried and barbecued (organoleptic and sensory characteristics), whereas snail meat had a significantly lower acceptance ($p<0.05$) among the respondents.

The protein, moisture, ash, fat and total carbohydrate and the mineral content of snail were determined by Felix (2013). The result shows that snail is rich in protein (82.96%) and low in both total carbohydrate (3.26%) and fat (3.98%). The major minerals found in this study were calcium (585.5 mg/100g) phosphorus (269.2mg/100g) potassium (331.8mg/100g) and zinc (39.0mg/100g). However iron and copper content of *A. Archatina* meat were less than 10mg/100g. This result revealed that the meat of *A. Archatina* is a good source of protein and minerals and may be used to treat nutritional deficiencies and manage disease such as high blood pressure, diabetes mellitus and stroke. It was evident from this study that Ar*chatina* meat is rich in protein and minerals but low in fats and its consumption can promote good human health.

Bukola and Abiodun(2011) studied the microbial quality of freshwater snail samples from Itu creek, Niger Delta Nigeria. The bacteria isolated were proteus sp, screptoccus. Pyrogens, shigella flexneri, staphylococcus aureus, E. coli, Webseilla aerogens, citrobacter, Bacillus subtilis, Bacillus cereus, Aeromonas sp, micro coccus liteus and vibrio cholera. Micrococuss liteus had the highest frequency of occurrence (10.25%). The fungi isolates were Aspergillus terreus, cladosporium sp, pusarium oxysporum, cryptococuss sp, and Aspergillus niger in which A. niger had the highest

occurrence (16.667%). The total microbial counts obtained from the work were found to be higher than the specified standard limits (1 x 10^5 cfu/g.) for bacteria and fungi 1x10^2cfu/g for coliforms. The crude protein content of the samples ranged from 28.87-33.41% in which the highest was obtained from A. fulica. The crude fat, crude fibre and carbohydrate content ranged from 1.8-4.25% and 00.10-0.18%, respectively. The mineral elements such Na, Ca, K and P were detected in all the freshwater snail samples. The concentration of Zn, Fe, Mn, Mg, Pb, As and Cu in the sample were 78.6-96.3mg/kg, 0.03-0.25 mg/kg, 0.04-0.37 mg/kg and 12.6-16.3 mg/kg respectively. It was found that the snails were nutritionally rich but harbor pathogenic micro-organism and heavy metals which can pose serious health hazard to consumers and they are not totally safe for human consumption.

Some aspect of the chemical and anatomical weight composition of land snails were analysed with a view to assessing the waste yield, carcass yield and their nutritional evaluation on wet weight basis by Adeyeye (2009). Proximate analysis of *Achachatina*, *Achatina* and *limicolaria* species was carried out on the carcass. Moisture and protein contents were high in all samples. Fat and ash contents were generally low, while crude fibre was not detected. The concentrations of iron, copper, zinc, manganese, sodium, calcium, potassium and cobalt were determined in the carcass. Values of iron, calcium, magnesium, sodium and potassium were consistently high while both chromium and cobalt were not detected. Anatomical fractionation showed the shell ton vary between 17.12 and 31.99%. Carcass varied between 36.97 and 45.14% and the intestine varied between 18.80 and 22.74%.

Jatto*et al*(2010), did a study on the proximate and mineral composition of four different species of snails shells and reported protein 0.12%, fibre 4.06%, fat 0.78%, ash content 2.00% for *Achatina*. Nitrogen free Extract (NFE) 93.04%, Zinc 9.83%mg/l, manganese 4.31mg/L, copper 6.47mg/L, and iron 251.23mg/L.*Achatinamarginata* had protein 0.42% fibre 3.37%, fat 0.75%, ash content 10.00%, NFE, 85.46%, Zinc 2.50mg/L; *Achatinafulica* had protein 0.30%, fibre 3.93%, fat 0.38%, ash content 10.00mg/L, NFE 82.36%, Zinc 8.02mg/L, manganese 16.98mg/L, copper 5.51mg/L, and iron 37.04mg/L; *limicolaria* species had protein 0.23% fibre 4.14%, fat 0.48%, ash content 13.00%, NFE 82.15%, Zinc 6.30mg/L, manganese 1.99mg/L, copper 4.46mg/L, and iron 208.58mg/L, the study shows the relevance of snail shell in water and waste water treatment.

Hamilton *et al*(2009)studied the mineral contents and heavy metal content of four predominant snail species in the Niger Delta: African land snail (*ArcchachtinaMarginta*) African Land snail (*Achatinafulica*), water snail (*pilaovata*) and garden snail (*limicolariaaurora*). The contents of K, Na, Ca, Mg, Zn, Fe and P were evaluated. The ranges of K, Na, and Ca contents of snail tissues were 40.4-61.7, 19.3-93.2 and 44.6-530.9 $\mu g/g$, respectively . ($p < 0.05$). Species differences were observed in the mineral contents. Higher concentration of K were recorded in *P.ovata*, A. *fulica* and *L.aurora* than in A. *marginata*. *L.aurora* tissues had ($p \leq 0.05$) higher Ca, Mg, and Zn than other snail tissues. Contents of Cu, Pb, Cd, Hg and Cr in the tissues were analyzed and compared with Federal Environment Protection Agency (FEPA) specifications for metals in foods. Cu, Pb, Cd, Hg and Cr were accumulated at levels lower than the World Health

Organization (WHO) and Federal Environmental Protection Agency (FEPA), Nigeria, specification for metal in foods.

The amino acids concentration of land snails *limicolaria* Sp, *Archatiriaarchatina* and *Archachatinamarginata*, were evaluated by Adeyeye and Afolabi (2003). Amino acid results showed that the protein contained nutritionally useful quantities of most of the essential amino acids including sulphur- containing amino acids. The total essential amino acids (TEAA) ranged from 361 to 45.0 mg/g crude protein (with histidine) while the TEAA without histidine ranged from331 to 403 mg/g crude protein. Significant differences existed between essential amino acids and non- essential amino acids at $p<0.05$ in A. *archatina* and A. *margmata*. Percentage total neutral amino acids ranged from 53.2 -62.0 mg/g crude protein; for total acidic amino acids, the range was 12.0 -25.4 and for total acidic amino acids the range was 18.8-26.0

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of samples

Three different species of snail were collected. The *Archachatina marginata* was collected from Edo State- Nigeria, *Archatina fulica* and *Limicolaria flammea* from Zaria, Kaduna State of Nigeria. A control sample which is an improve variety of the species of *Archachatina marginata* was collected from Delta State of Nigeria. A total of twenty (20) samples consisting of ten (10) eachof*Limicolariaflammea*and *Archatina fulica* species. In the same vein,20 samples of*Archachatina marginata wild and farmed* species (10)each were collected from the southern region of the country (Edo and Delta States). The samples from Edo and Delta States were obtained and analyzed during the dry season, while those from Zaria-Kaduna State was analyzed during the raining season.

3.2 Preparation Of Sample

The flesh of the snail was carefully removed from the shell and oven dry at 90^0C. The sample was crushed and sieved using a 0.4mm mesh(Gary, 2004). This was used for the digestion and proximate determinations.

3.3 Digestion Of Snail Sample

One gram of the sample was weigh into a 200 cm^3 Kjeldehl Mask and 20ml of digestion mixture (1:1 mixture of 8M HCl 8MHNO_3) was added.The mixture was allowed to stand for 24 hours before digestion was done under reflux for 45 minutes. The sample was heated a little to remove the brown fumes of NO_2.The solution was then allowed to cool and made up to 50cm^3 with distilled water and then stored in a treated polythene bottle for AAS analysis (Onianwa2001).

3.4 Proximate Analysis Procedure

3.4.1 Moisture Determination Using Oven Method Procedure

The plastic dishes were washed thoroughly and dried in oven, and were placed inside a desiccators to cool and weighed.Samples werethen placed on the weighed dishes and the weight of the dishes plus weight of the un-dried samples taken (induplicate).The sampleswere allowed to dry in the oven for two hours at between 90^0C – 105^0C, and thenat 100 – 135^0C for the next 4 hours until constant weight was obtained. The sample was put in the desiccators and allowed to cool, and the dry weight of the sample plus the dish was taken. The calculation for the moisture was done as follows.

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = initial weight of empty dish,

W_2 = weight of dish + food before drying

W_3 = final weight of dish + food after drying

% total solid (drymatter) = 100 - %moisture. AOAC.(1980).

3.4.2 Ash content determination

Dry sample of finely ground snail was weigh into a crucible and the sample was then transferred into a pre-heated muffle furnace at 550^0C, and left at that temperature for 2 hours until a white or light gray ash was obtained. Finally it was cooled in a desiccator and reweighed.The percentage ash sample was calculated as follows:

% Ash (dry basis) = $\frac{W_3 - W_1}{W_2 - W_1} \times 100$

Where:

W_1 = weight of empty crucible,

W_2 = weight of crucible + food before drying and/or ashing

W_3 = weight of crucible + ash (AOAC 1980).

3.4.3 Crude Fibre

Crude fibre represents the organic residue left behind after the material has been treated under standardized conditions with light petroleum, boil with dilute sulphuric acid, dilute sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether.Crude fibre in made largely of cellulose together with a little lignin. Crude fibre includes, theoretically, materials that are indigestible in human and animal organism.Henneuberg, *et al.*, (1864) developed the most popular method of determining fibre presently being used today.

The samples weredefatted using petroleum ether (If the fat content is more than 10%). It wasboiled under reflux for 30 minutes with 200 cm^3 of a solution containing 1.25g of H_2SO_4 per 100 cm^3 solution.The solution was filtered through linen on a fluted funnel and washed with boiling water until the washings are no longer acidic to litmus paper. The residue was transferred to a beaker and boiled for 30 minutes with 200 cm^3 solution containing 1.25g of carbonate free NaOH per 100 cm^3.The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible.The residue was dried in an electric oven and weighed, incinerated, cool and

weighed. The loss in weight after incineration x 100 is the percentage of crude fibre(AOAC1980).

3.4.4 Determination of Fat

Lipids are characterized by their sparing solubility in water and their considerable solubility in organic solvents. Determination of fat content of a food does not actually reflect the estimation of the fat content, but of the lipid fraction of the food that is, those food constituents soluble in non – polar organic solvents such as benzene and petroleum ether. The lipid fractionsthat include fats are also known as triglycerides or triglycerols. In real term, fat make upto about 99% of the lipid fraction of a food. Usually in food analysis total lipid content is what is determined rather than the true fat content and this has resulted in the terms fat and lipid becoming virtually indistinguishable. Most common method used for fat determination is soxhlet extraction method.

250 cm^3 clean boiling flasks were dry in oven at 105 – 110^0C for 30 minutes. It was transferred to a desiccator and allowedto cool. Samples were placed into labeled thimbles and boiling flask was filled with 300 cm^3 of petroleum ether (boiling point 40 – 60^0C). The extraction thimble was plug lightly with cotton wool. The soxhlet was assembled and allowed to stand for 6 hours.Petroleum ether was removed and the samples dried at 105^0 – 110^0C for 1 hour. It was transferred from oven into a dessicator and allowed to cool, then weighed (AOAC, 1980).

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$$\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3.4.5 Determination ofProtein

Amino acids are the building blocks of proteins. Proteins are therefore polymers of amino acids most of which are α – amino acids having the general formula NH_2CHR_2COOH. It is the only macronutrient in foods that contains nitrogen. The nitrogen in proteins becomes the basis of the estimation of protein in foods.

Designated weight of a given food is digested with concentrated H_2SO_4 (an oxidizing agents); anhydrous sodium sulphate (just to increase the boiling point) and a catalyst such as copper, titanium, selenium or mercury. This treatment converts the nitrogen component in the food (except nitrate and nitrite nitrogen), into ammonium sulphate:

$$N\ (food) \rightarrow (NH_4)_2\ SO_4$$

The resistance ammonium sulphate is converted to ammonia gas by heating with sodium hydroxide in the presence of steam.

$$(NH_4)_2\ SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2 + 2NH_3$$

The ammonia generated is collected in an excess of boric acid:

$$2NH_3 + 2H_3BO_3 \rightarrow 2NH_4H_2BO_3$$

Subsequently the nitrogen is estimated by titration of the ammonium borate produced with standard H_2SO_4 or HCl that is

$2NH_4H_2BO_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4 + 2H_2BO_3$ when

1 mole H_2SO_4 = 2 moles N = 28gN

Or 1 mole HCl = 1 mole N = 14gN

Thus 1ml 0.1MH_2SO_4 = 0.0028gN

1ml 0.1MHCl = 0.0014gN

The Procedure for Crude Protein is as follows:

2g of sample was weighed and place in 600 cm^3 Philips conical flask.1g pure scale pepsin was dissolved in 490 cm^3 distilled water and sample was added. 10 cm^3 of 25% HCl (by weight) was added and the beaker and contents place in incubator (37 – 40^0C).At the end of exactly 24 hours, further 10 cm^3 of acids was added, stir well and return to incubator.At the end of second 24 hours, it was filter and wash until free from acid. The filter paper and residue was transferred to Kjeldahl flask and nitrogen was determined.

Using HCl

$$\% \text{ Nitrogen} = \frac{V_S - V_B \text{ x Nacid x } 0.01401 \text{ x } 100}{W}$$

Where:

V_s = vol (ml) of acid required to titrate sample

V_B = vol (ml) of acid required to titrate blank

W = wt of sample in grams

Nacid = normality of acid (0.1N)

% crude protein = N x (conversion factor) (100/% nitrogen in protein) = conversion factor

% digestibility crude protein = total crude protein minus - % crude protein in filter paper residue(AOAC,1980).

3.4.6 Determination ofCarbohydrates

Carbohydrates are generally the most abundant singular food component in nature and are widely distributed. Carbohydrates are classified into

i. Monosaccharides, examples of which are xylose, arabinose, glucose and fructose

ii. Oligosaccharides which occur when the hydroxyl group of one monosaccharide is condensed with reducing end of another monosaccharide (two sugars when joined in this way produces, a disaccharide).If however a linear array of three to eight monosaccharide join in this manner an oligosaccharide is generated.

iii. Polysaccharides which are grouped into two–the structural polysaccharides (which include cellulose, hemicelluloses, lignin). They constitute the rigid, mechanical structures in plants and nutrient polysaccharides (include, starch, glycogen) which can easily be digested.

Essentially, methods of carbohydrates analyses are by difference, titration, colorinmetry refractometry, polarlimetry, enzymes methods and High Performance Liquid Chromatography (HPLC).

Procedure for carbohydrates by difference is used. In this method, carbohydrates content is obtained by calculation having estimated all the other fractions by proximate analysis.

% avalable carbohydrates = 100 – (% moisture + % ash + % protein + % fibre).

The carbohydrates content is usually given as total carbohydrates by difference that is the percentage of water, protein, fat and ash subtracted from 100.

3.4.7 Determination ofOil

1g of sampleswas placed into a conical graduates centrifuge tube. The sample was thoroughly mixed with 10 cm^3 distilled water for 30 seconds. The sample was allowed to stand for 30 minutes at room temperature and then centrifuge for 30 minutes. The volume of free oil (the supernatant were read directly from the graduated centrifuge tube. The amount of oil absorbed (total minus free) were multiplied by its density for conversions to grams(AOAC, 1980).

3.4.8 Amino Acid Profile

The Amino Acids in thesamples were determined using methods described by Benitez, (1989). The known sample were dried to constant weight, defatted using chloroform / methanol mixture of ratio 2:1. About 4g of the sample were put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus(AOAC, 2006), then hydrolyzed with known weight of the defatted sample into glass ampoule. 7 cm^3 of 6N/HCl was added and oxygen expelled by passing nitrogen into the ampoule (This was done to avoid possible oxidation of some amino acids such as methionine and cystineduring hydrolysis). The glass ampoule was then scaled with Bunsen burner flame and put in an oven preset at 105 $C^o \pm 5^0C$ for 22 hours. The ampoule were allowed to cool before broken open at the tip and the content was filtered to remove the remains.The filtrate was then evaporated to dryness in hot air oven. The residue was dissolved with 5

$cm^3$of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in freezer. 5 to 10microlitreloaded into the tecnicon sequential multi- sample Amino Acid Analyzer (TSM). This was dispended into the cartridge of the analyzer. The TSM analyzer is desired to separate and analyze three acidic, neutral and basic amino acids of the hydrolysis. The analysis lasted for 76 minutes. An integrator attached to the analyzer calculated the peak area proportional to the concentration of each of the amino acids. Alternatively, the net height of each peak produced by the chart recorder of TSM was measured. Approximately area of each peak was then obtained by multiplying the height with the width at half – height.The norclaucine equivalent (NE) for each amino acid in the standard mixture were calculated using the formula.

$$NE = \frac{\text{Area of Norceucine Peak}}{\text{Area of each Amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std}$ x Molecular weight x µMAA S_{td}

Finally, the amount of each amino acid present in the sample was calculated in g / 100 g protein using the following formula.

$$\text{Concentration (g/100 g protein)} = NH \times W@\ NH/2 \times S_{td} \times C$$

$$\text{Where } C = \frac{\text{Dilution x 16}}{\text{Sample wt (g1 x N\% x 10 x Vol Loaded}}$$

Where NH = Net Height

W = Width @ half height

nleu = Nor leucine

CHAPTER FOUR

4.0 RESULTS:

Figure. 4.1 is a pie chart, representing the fours colour of snail samples of*Archachatina marginata, Archatinafulica,Limicolariaflammea* and control (improved *Archachatinamarginata).* (AM, AF, LF and C).

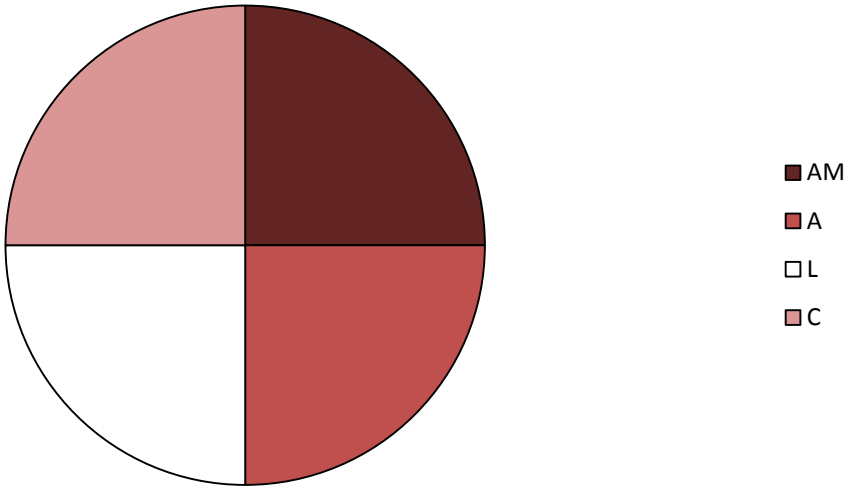


Figure 4: 1: Colour Appearance of Snail Sample

Species	**Appearance**
AM	Dark brown
AF	Light brown
LF	Whitish brown
C	Brown

Where AM represent *Archachatina marginata*, A represent *Archatina fulica* species, L represent *Limicolaria flammea* species and C represent the controlled sample. The texture of the three snail samples found during the study, is presented in Table 4.1.

Table 4.1**:**Physical Properties of Snail Samples (Texture)

Species	**Appearance**
AM	Smooth skin
AF	Rough skin (very)
LF	Smooth skin
C	Smooth skin

Where AM, AF, LF and C represent *Archachatina marginata*, *ArchatinafulicaLimicolariaflammea*and controlled sample respectively.

Figure 4.2shows the heavy metal concentration (mg/kg) of*Archachatina marginata*, *Archatina fulica* and *Limicolaria flammea*, and control.

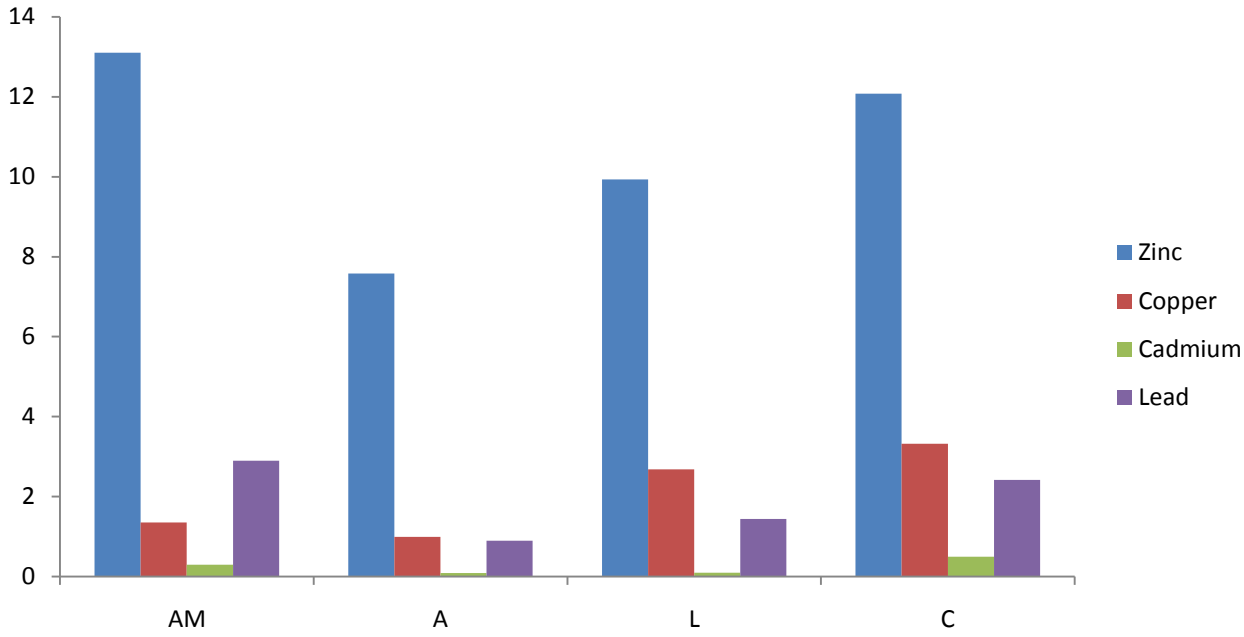


Figure 4: 2: Concentration of zinc, copper, cadmium and lead in snail Samples.

Where AM, AF, LF and C represent *Archachatina marginata, fulicaArchatina, Limicolaria mammea* and control sample respectively.

Figure 4.3present the proximate composition, the moisture, ash and crude protein content of *Archachatina marginata*, *Archatina fulica* and *Limicolaria flammea*, and control species.

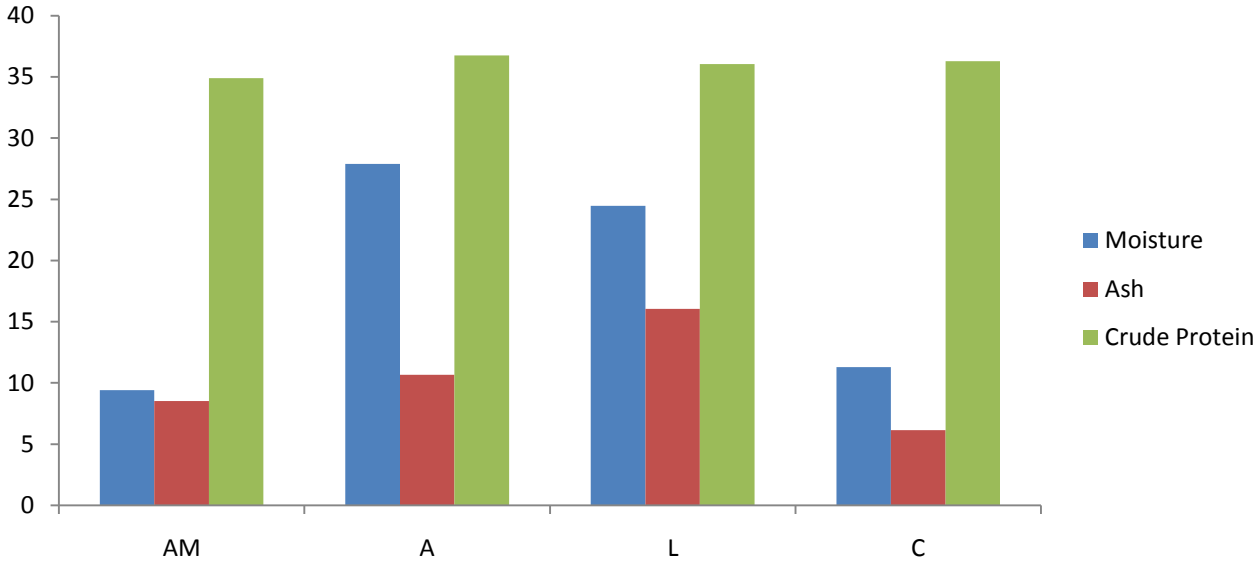


Figure 4.3: Percentage Composition of Moisture, Ash and Crude Protein in the Samples

Where AM, AF, LF and C represent *Archachatina marginata*, *Archatina fulica* *Limicolariaflammea*and control sample respectively.

Figure 4.4shows the crude fiber, crude lipid and carbohydrate composition of four snails samples*Archachatina marginata, Archatina fulica* and *Limicolaria flammea*, and control.

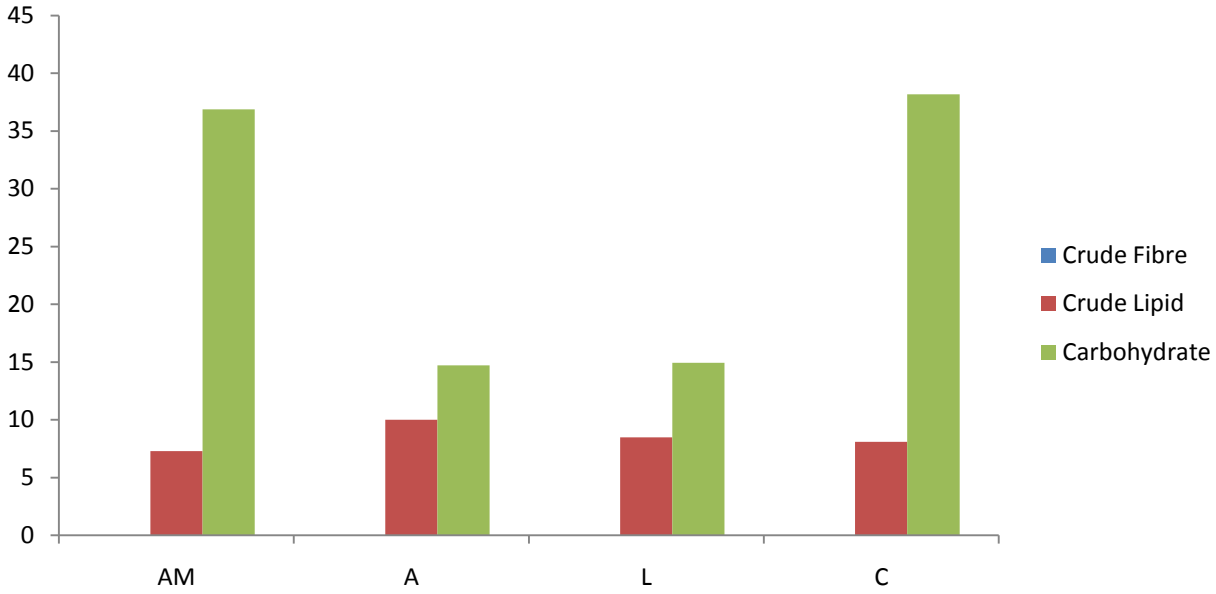


Figure 4: 4:Percentage Composition of Crude Fibre, Crude Lipid and Carbohydrate in Snail

AM, AF, LF and C represent *Archachatina marginata, Archatina fulica Limicolariaflammea*and control sample respectively.

Figure 4.5 shows theAmino Acid profile of*Archachatinamarginata*,*Limicolaria flammea*, *Archatinafulica* and control sample

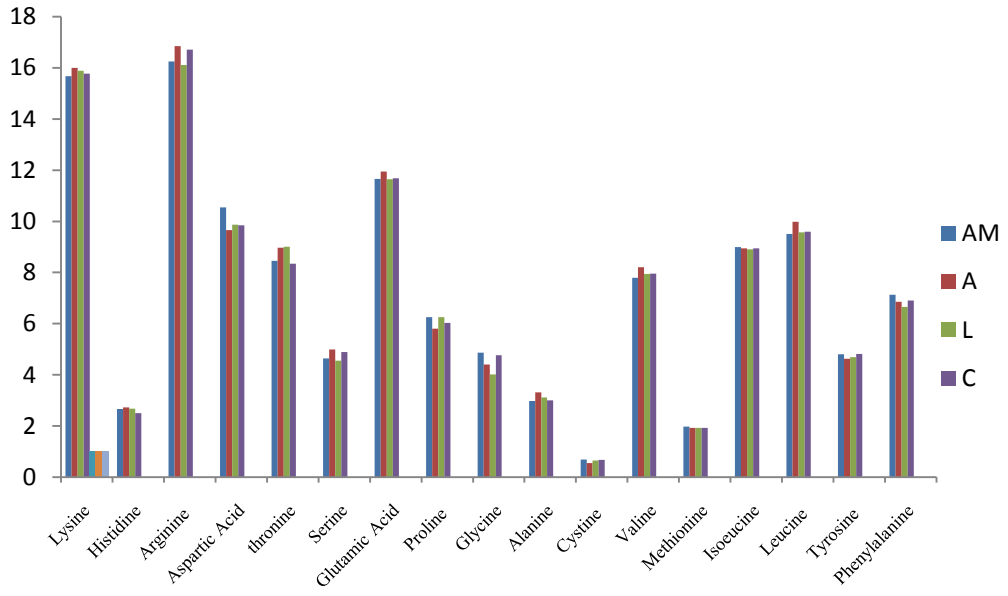


Figure 4: 5:Amino acid profile of the snail samples.

Where AM represent *Archachatina marginata* AF – *Archatinafulica*, LF – *Limicolaria flammea* and C – control sample.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Properties Of The Snail

The snail, *Archatina fulica,*clearly shows a rough reticulated texture, unlike the other species with smooth texture like the *Archachatina marginata* and *Limicolaria flammea*, it is worth wetting the snail before taking a picture as this helps to bring out the colour and texture much better.

5.2 Heavy Metals Level Of Sampled Snail

5.2.1 Zinc

The mean concentration of zinc in snail sample were 13.10 ± 0.03 (mg/kg), 7.58 ± 0.03 (mg/kg), 9.93 ± 0.04 (mg/kg) and 12.08 ± 0.02 (mg/kg) in *Archachatina marginata*, *Archatina fulica*, *Limicolaria flammea* and control sample respectively (Figure 4.2). All the species samples shows significant difference between them.The level of Zn is within the permissible limits of (WHO) which recommend a daily intake for Zn to be 2.3 g for Adult of 70kg (WHO, 1993). The level of Zn in the snails depends on the environment, requirement of the area the snail grows, and what it feed on. Zinc is needed by human and other animals in small amount, but in larger amount can cause damage to the nervous system, fatigue and irritability. These health problems include hair and finger nail loss, damage to kidney and liver tissue. These concentration is lower than the one obtained by Ezemonye, *et al.*(2009), with a zinc concentration of 35.35 – 77.09mg/kg. These can be attributed to the environment where the samples were obtained and method of handling and processing the sample.

5.2.2 Copper

The mean concentration of copper (Cu) in the snail samples were 1.35 ± 0.01mg/kg, 0.99 ± 0.01mg/kg, 2.68 ± 0.03mg/kg and 3.32 ± 0.01mg/kg for AM, AF, LF and C respectively. The four samples show significant difference at 95% and 90% confidence limit. The maximum contaminant level goal (MCLG) for copper is 1.3mg/l (EPA, 2007). EPA has set this level of protection based on the best available science to prevent potential health problem. These concentration are low when compared to result obtained from fresh water snail by Ezemonye, *et al*. (2010) with the value of copper 4.56 – 671µgg^{-1}. Copper is an essential substance in human life, but in high doses, it can cause anemia, liver and kidney damage Lenntech, (1998). The level of copper can be attributed to the environment and kind of waste deposited in the area.

5.2.3 Cadmium

The mean concentration of cadmium (Cd) were 0.30 ± 0.00mg/kg, 0.09 ± 0.00mg/kg, 0.10 ± 0.00mg/kg, and 0.50 ± 0.00mg/kg for AM, AF, LF and C respectively. The control sample had the highest value of Cd. The permissible limit for cadmium is 1.0 mg/kg with a daily intake estimated as 0.008mg to 0.001 mg/kgbody weight per day. This is equivalent to 0.07 mg per day for a 70 kg adult(WHO, 1993). Cadmium is a dangerous element because it can be absorbed via the alimentary track; penetrate through placenta during pregnancy and damage membrane and DNA. These values obtained in this work are lower when compared with that of Adedeji*et al.,* (2010)with the cadmium level at 0.71mg/kg. These values obtained can be attributed to the habitat and activities found in and around the region, and the soil level of pollution.

5.2.4 Lead

The mean concentration of Lead (Pb) in snail sample were found to be 2.90 ± 0.00mg/kg 0.90 ± 0.00mg/kg, 1.44 ± 0.01mg/kg and 2.42 ± 0.03mg/kg for AM, AF. LF and C respectively. The values are significantly different from each other, with AM species having the highest concentration of lead.Lead has a permissible limit of 0.5mg/kg and a value of 0.245 mg per day for a 70/kg adult (WHO, 1993). These values are higher when compared with that of Adedeji and Adeyemo (2010), which has lead concentration in snail sample at 0.24mg/kg. Human exposure to lead can result in a wide range of biological effect depending on level and duration of exposure; effects occur over a broad range of closes, with the developing foetus and infants being more sensitive than the adult. Average daily lead intake for adults in the UK is estimated at 1.6mg from air, 20/mg from drinking water and 28/mg from food, Lenntech, (1998). The higher values of lead in all samples can be attributed to level ofpollution of the habitat.

5.3 Determination Of Proximate Composition Of TheSnails Species

5.3.1 The Moisture Content Samples

Moisture content of food is of great importance to every food processor as a number of biochemical reactions and physiological changes in food depend very much on the moisture content. Of even greater significance is the effect of moisture on the stability and quality of foods.

The moisture content of AM, AF, LF and C were 9.14%, 27.89%,24.47% and 11.30% respectively. The improved or farmed sample had 11.30 – 11.44% which is generally lower than that of *Archatina fulica* and *Limicolaria flammea* species indigenous to the Zaria. The highmoisture content in the two species *Archatinafulica*and *Limicolaria flammea*could be attributed to the fact that it was obtained during the rain(wet season), and so the moisture content is expected to be higher than the *Archachatina marginata* and the improved species which were harvested during the dry season from the southern part of the country. The moisture content values are lower when compared to the Ademolu *et al*(2004), who reported a moisture content of *Archachatina marginata*as 76.56% ± 0.04% and *Limicolariaspp.* As 78.68% ± 0.68% respectively.

5.3.2 The Crude Lipid Content

The percentage of crude lipid in snail species were as follows 7.29 ± 0.63%, 9.99 ± 0.15%, 8.47 ± 0.79% and 8.09 ± 1.11% for AM, AF, LF and C respectively. The improved sample (C)crude lipid content falls between that of AM and LFspecies. These results show a significant difference in values at90% confidence level in all species. Like other food sources, snails do contain fat although not very much. An entire serving contains just 1 – 4grams of fat (Adeola, 2010). This result of lipid content is greater thanthat reported by Jessica and Bernard (2003), which ranges from 2.0 ± 0.4 to 0.9 ± 0.1 (mean ± SE)mg/kg.

5.3.3 The Ash Content Of The Snails Species

The ash content was 8.53 ± 0.63%, 10.66 ± 0.49%, 16.06 ± 0.75 and 6.15 ± 0.99%, for AM, AF, LF and C respectively. Species LF and AF showed no significant difference at ($p>0.05$) while the rest showed significant differenceat ($p<0.05$) respectively. The relatively high values of ash content implies that they may have a reasonable quantity of mineral elements for building healthy body and proper functioning of body tissues. Ash content is a measure of the total amount of mineral present within a given sample of food, whereas mineral content is a measure of the amount of inorganic compound present within a given food(Nelson, 2002).

According to James (1996), mineral elements are classified on the following basis:

i. Major elements (>0.01% or 100ppm): calcium, phosphorus, chlorine, sulphur, potassium, magnesium and sodium.

ii. Trace elements (< 0.01% or 100ppm): arsenic, copper,, iron, nickel, tin, chromium, fluorine, manganese, selenium, vanadium, cobalt, iodine, molybdenum, silicon and zinc.

iii. Non – essential and toxic mineral elements: beryllium, lead, cadmium, palladium, mercury and thallium.

The ash content values are higher when compared to the work of Ademolu *et al.*, (2004) where the Ash content in *Archachatina marginata* (AM) and *Archatinafulica* (AF) was 1.44% ± 0.01% and *Limicolaria flammea (*LF) was 1.34% ± 0.02%.

5.3.4 The Crude Protein Of Snails Species

The range of crude protein contents in the snail sample analysis was 34.90 ± 0.23%, 36.75 ± 0.22%, 36.06 ± 0.17% and 36.28 ± 0.41% for AM, AF, LF and C respectively. It showed no significant difference in AF, LF and C within confidence interval of 95.00% but did with AM species. Malik, *et al*. (2010) reportedcrude protein of other meat as 18.3% in chicken, 20.0% in rabbit, 19.6% in beef and highest in snail meat sample with 20.5%. This shows that snail meat is richer in protein. Most of the calories in a dish of snails come from protein. A 100 g serving adds 16.5grams of protein to a diet, compared to 50 g in a serving of white meat chicken and 25 g dark – meat chicken.

5.3.5 The Crude Fibre Of the Snails Samples

No crude fibre was detected in any of the snail samples species. These resultsare in aggrement with that of Ademolu *et al*., (2004). Low crude fibre content can be attributed to a decrease in microbiological metabolism of lingo polysaccharide or due to non-utilization of the sugar during metabolic activities leaving a lower or no fibre content (Oshodi and Ekperigin, 1987). It also shows that snails can favourably be compared with seafoods which are completely absorbed by the body.

5.3.6 The Carbohydrate Content In The Snail Samples

The carbohydrate content of the snails samples are 36.87 ± 0.31%, 14.71 ± 0.17%, 14.94 ± 5.02% and 38.18 ± 0.66% for AM, AF, LF and C respectively.Samples of *Archatina fulica* and *Limicolariaflammea*showed no significant difference at ($p > 0.05$) confidence limit, while *Archachatina marginata* and control shows significant difference at ($p < 0.05$) level. It shows that AF and LFare low in carbohydrate and the highest valueof carbohydrate was found in the control sample. This is also in accordance with Ademolu(2004). If one is following a low carbohydrate diet, snails fits well into your diet as a snack, an appetizer or a meat. A serving of snails contains just 2grams of carbohydrate while the butter does not add a single carbohydrate Adeola *et al*., (2010).

5.4 The Amino Acid Profile Of The Snail Samples

The amino acid profiles of the samples are shown inFigure 4.5. It indicates that all the essential amino acid required by an individual are present, even with the non – essential and all fall within the recommended limit by WHO (2007). Ammonia and Norlaucine were not detected.

Lysine in *Archachatina marginata* was 15.6 $\pm$ 0.14 g/mg protein,*Archatina fulica* was16.00 $\pm$ 0.10g/mg protein, *Limicolaria flammea* had 15.89 $\pm$ 0.35 g/mg, and control sample was 15. $\pm$ 0.33 g/mg. The daily amount by WHO is 30 mg/kg in 70 kg body weight.Histidine was 2. $\pm$ 0.03 g/mg for AM, and AFwas 2.3$\pm$ 0.00 g/mg. Leucine shows AM 9.51 $\pm$ 0.5, AF 9.98 $\pm$ 0.43 LF 9.57 $\pm$ 0.07 and C 9.60 $\pm$ 0.2 g/mg.The WHO daily amount is 39 mg/kg in 70kg body weight. Phenylalanine show AM 7.13 $\pm$ 0.12, AF 6.86 $\pm$ 0.10, LF .6 $\pm$ 0.30 and C.91 $\pm$ 0.38 g/mg. The WHO daily amount is 25 mg/kg in 70kg body weight. Valine was AM 7.79$\pm$ 0.11, AF 8.21 $\pm$ 0.09, LF .94 $\pm$ 0.14 and C 7.95 $\pm$

0.06 g/mg. WHO daily amount is 26 mg/kg in 70 kg body weight.Isoleucine was AM 9.00 ± 0.14,AF 8.94 ± 0.07, LF 8.91 ± 0.23 and C 8.94 ± 0.06 g/mg.The WHO daily amount is 20 mg/kg in 70 kg body weight. Methionine AM 1.98 ± 0.02, AF 1.93 ± 0.00, LF 1.93 ± 0.00 and C 1.93 ± 0.00 g/mg. WHO daily is 10.4 mg / kg in 70kg body weight. Cystine AM 0.69 ± 0.00, AF 0.55 ± 0.03, LF 0.5 ± 0.01 and C 0.68 ± 0.07 g/mg, and WHO is 10.4 mg/kg in 70 kg of body weight. Tyrosine AM 4.80 ± 0.10, AF 4.63 ± 0.35, LF 4.9 ± 0.02 and C 4.81 ± 0.07 g/mg, and the WHO daily amount is 25 mg/kg in 70 kg body weight. Thronine shows AM 8.46 ± 0.10, A F8.97 ± 0.09, LF 9.01 ± 0.31 and C 8.34 ±0.22 g/mg and WHO daily amount is 15 mg/kg in 70kg body weight. Alanine, Arginine, Proline and Serine are non – essential and had no WHO daily amount limit.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1. Summary

The mean concentration for zinc content in snail sample was 13.10 ± 0.03mg/kg for *Archachatina marginata*, 7.58 ± 0.03mg/kg for *Archatina fulica*, 9.93 ± 0.04mg/kg for *Limicolaria flammea* and 12.08 ±0.02mg/kg for the control sample. *Archachatina marginata* have the highest level of Zinc content in this work done and *Archatina fulica* with the least.

The mean concentration of copper content in snail was 1.36 ± 0.01mg/kg for *Archachatina marginata*, 0.99 ± 0.01mg/kg for *Archatina fulica*, 2.68 ± 0.03mg/kg for *Limicolaria flammea* and 3.32 ± 0.01mg/kg for the control sample, the control has the highest copper content in this work; the daily intake is 1.3mg/l. The values are within the permissible range by EPA, (2007).

The mean concentration of cadmium in snail was 0.30 ± 0.00mg/kg for *Archachatina marginata*, 0.09 ± 0.00mg/kg for *Archatina fulica*, 0.10 ± 0.00mg/kg for *Limicolaria flammea* and 0.50 ± 0.00mg/kg for control which is the highest level of cadmium found in snail in this work. WHO (1993) permissible limit for cadmium is 1.0mg/kg.

The mean concentration of lead in snail was 2.90 ±0.00mg/kg for *Archatina fulica*, 1.44 ± 0.01mg/kg for *Limicolaria flammea* and 2.42 ± 0.03mg/kg for the *archachatinamarginata* had the highest level of lead. The WHO permissible limit for lead is 0.5mg/kg.

It was observed that the species from the South has more of the heavy metal when compared to the species in the North.

The crude protein content was 34.90 ± 0.23% for AM, 36.75 ± 0.22% (A) 36.06 ± 0.17% (L) and the control (C) having 36.28 ± 0.41%. The ash content was 8.53 ± 0.63% for (AM) *Archachatina marginata*, 10.66 ± 0.49% for (A) *Archatina fulica*, 16.06 ± 0.75% for (L) *Limicolaria flammea*, and 6.15 ± 0.99% for control which was the least and *Limicolaria flammea* the highest.

The crude lipid content was 7.29 ± 0.63% for *Archachatina marginata*, 9.99 ± 0.15% for *Archatina fulica*, 8.47 ± 0.79% for *Limicolaria flammea* and 8.09 ± 1.11% for the control sample. The carbohydrate content was 36.87 ± 0.31 for *Archachatina marginata*, 14.71 ± 0.17% for *Archatina fulica*, 14.94 ± 5.02 for *Limicolaria flammea* and control has 38.18 ± 0.66% as the highest. The moisture content was 9.41 ± 0.33% for *Archachatina marginata*, 27.89 ± 0.52% for *Archatinafulica* 24.47 ± 0.17% for *Limicolaria flammea* and 11.30 ± 0.41% for the control sample. The crude fibre content was not detected in any of the species; this indicated a satisfactory level of mineral content in snail and a good balance diet for both young and old. The low moisture content determined in *Archachatina marginata* and control could be attributed to the season the research was carried out, that is the, dry season. The non – detection of fibre also shows that snail can be absorb completely in the body and it can be compared only with seafood.

Archatina fulica has the highest level of crude protein 36.75 ± 0.22% and *Archachatina marginata* with the least 34.90 ± 0.23% which is still good and high when compared to other red meat and chickens. Snail's samples from the southern region used

for this research tend to be higher in heavy metal concentration when compared to the snail samples from the Northern region of Nigerian.There was no much significance difference between the snails picked from the field (wild) *Archatina fulica*, *limicolaria* and *Archachatinamarginata* and the farmed (improved) specie of *Archachatinamarginata.* The improvedspecie (C) was also high in carbohydrate witha value of 38.18%. These could be attributed to the type of soil and season in the region where it is farmed.

6.2 Conclusions

Archachatina marginata, *Archatina fulica* and *limicolaria flammea*have been analysed for their heavy metals content, to ascertain its safety for consumption. The values obtained were within the tolerable limit of daily consumption set by WHO, (1993).

The mean concentration of zinc ranged from $7.58^{\pm}0.03$ to $13.10^{\pm}0.03$ mg/kg while copper ranged from $0.09^{\pm}0.00$ to $0.50^{\pm}0.00$mg/kg. The mean concentration for lead in all samples ranged from $0.90^{\pm}0.00$ to $2.90^{\pm}0.00$mg/kg. The permissible limit for is 0.5mg/kg in 70kg adult (WHO, 1993).

Based on the high percentage of crude protein content in snail meat, the meat serves as a good alternative source of protein to the body. Adequate consumption of snail meatwould help to alleviate deficiency of protein in the populace. The results also shows that the samples are low in fat and calories and hence can be absorbed completely by the body of human.

6.3 Recommendation

The following recommendations are therefore made.

i. Further study should be carried out to investigate the level of heavy metals the habitat of the snail and correlate this with the heavy metal level of snail flesh.

ii. Investigation should be extended to other species of snail.

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APPENDIX A

Concentration of Heavy Metals in (mg/kg) of 3 Species of Snail Samples and Control *Archachatina marginata, Archatina fulica* and *Limicolaria flammea*, and control which is shown in the table below:

Snail species	**Zn(mg/kg)**	**Cu(mg/kg)**	**Cd(mg/kg)**	**Pb(mg/kg)**
AM	13.10 ± 0.03	1.35 ± 0.01	0.30 ± 0.00	2.90 ± 0.00
AF	7.58 ± 0.03	0.99 ± 0.01	0.09 ± 0.00	0.90 ± 0.00
LF	9.93 ± 0.04	2.68 ± 0.03	0.10 ± 0.00	1.44 ± 0.01
C	12.08 ± 0.02	3.32 ± 0.01	0.50 ± 0.00	2.42 ± 0.03

Where AM, AF, LF and C represent *Archachatina marginata, Archatina fulica Limicolaria flammea* and control samples respectively.

APPENDIX B

Proximate Composition of Snails (% mean ± SD)

In *Archachatina marginata*, *Archatina fulica* and *Limicolaria flammea*, and control which is shown in the table below:

Composition	*Archachatina Marginata*	*Archatina Fulica*	*Limicolaria Flammea*	*Archachatina Marginata* (Farmed)
Moisture	9.41 ± 0.33	27.89 ± 0.52	24.47 ± 0.17	11.30 ± 0.41
Ash	8.53 ± 0.63	10.66 ± 0.49	16.06 ± 0.75	6.15 ± 0.99
Crude Protein	34.90 ± 0.23	36.75 ± 0.22	36.06 ± 0.17	36.28 ± 0.41
Crude fibre	0.00	0.00	0.00	0.00
Crude Lipid	7.29 ± 0.63	9.99 ± 0.15	8.47 ± 0.79	8.09 ± 1.11
Carbohydrate	36.87 ± 0.31	14.71 ± 0.17	14.94 ± 5.02	38.18 ± 0.66

APPENDIX C

The Amino Acid profile of the four snails samples(g/100g protein).

S/No	Amino Acid	AM	AF	LF	C
1	Lysine	15.67± 0.14	16.00± 0.10	15.89± 0.35	15.77± 0.33
2	Histidine	2.67± 0.03	2.73± 0.00	2.68± 0.01	2.50± 0.00
3	Ammonia				
4	Arginine	16.25± 0.43	16.85± 0.14	16.11± 0.10	16.71± 0.30
5	Aspartic acid	10.54± 0.01	9.66± 0.0.05	9.87± 0.70	9.84± 0.67
6	Thronine	8.46± 0.10	8.97± 0.09	9.01± 0.31	8.34± 0.22
7.	Serine	4.64± 0.11	4.99± 0.01	4.55± 0.08	4.89± 0.50
8.	Glutamic acid	11.66± 0.17	11.94± 0.72	11.64± 0.00	11.68± 0.69
9.	Proline	6.26± 0.38	5.80± 0.27	6.26± 0.10	6.03± 0.21
10.	Glycine	4.86± 0.40	4.40± 0.07	4.01± 0.44	4.77± 0.60
11.	Alanine	2.98± 0.42	3.31± 0.03	3.11± 0.07	3.00± 0.01
12.	Cystine	0.69± 0.00	0.55± 0.00	0.65± 0.00	0.68± 0.00
13.	Valine	7.79± 0.11	8.21± 0.09	7.94± 0.14	7.95± 0.06
14.	Methionine	1.98± 0.02	1.93± 0.00	1.93± 0.00	1.93± 0.00
15.	Isoeucine	9.00± 0.14	8.94± 0.07	8.91± 0.23	8.94± 0.06
16.	Leucine	9.51± 0.56	9.98± 0.43	9.57± 0.07	9.60± 0.62
17.	Norleucine				
18.	Tyrosine	4.80± 0.10	4.63± 0.35	4.69± 0.02	4.81± 0.01
19.	Phenylalanine	7.13± 0.12	6.86± 0.10	6.66± 0.30	6.91± 0.38

Where AM represent *Archachatina marginata* AF – *Archatina fulica* LF – *Limicolaria flammea* and C – control sample.