CHEMICAL COMPOSITION, NUTRIENT BIOAVAILABILITY AND CONSUMER ACCEPTABILITY OF *CIRINA FORDA* (WESTWOOD) LARVA-ENRICHED VEGETABLE SOUPS

DABOH, OLADELE OLATUNJI

Matriculation Number: 147613

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CERTIFICATION

I certify that this research titled "Chemical Composition, Nutrient Bioavailability and Consumer Acceptability of *Cirina forda* (Westwood) Larva-enriched Vegetable Soups ''was carried out by Daboh, Oladele Olatunji in the Department of Human Nutrition and Dietetics, Faculty of Public Health, College of Medicine, the University of Ibadan under my supervision.

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(Supervisor)

Dr. Oladejo Thomas Adepoju

Department of Human Nutrition and Dietetics,

University of Ibadan

ABSTRACT

One of the public health concerns in developing countries is still protein-energy malnutrition and micronutrient deficiency. Some edible insects, especially *Cirina forda* (CF) larvae with high protein, minerals, and vitamins could serve as a source of these nutrients in traditional foods in most localities. However, there has been a steady decline in consumption of CF due to fear of toxicity and deforestation; hence, the need to investigate the nutrient bioavailability and food safety of the edible larva. This study examined the consumption pattern, nutrient bioavailability, toxicology, nutrient composition, and consumer acceptability of CF larva-enriched vegetable soups.

The cross-sectional design with an analytical component was adopted. Respondents which comprised five hundred and two from two urban and three rural wards in Iseyin and Saki East Local Government Areas of Oyo State (where the insect consumption was popular) were recruited through a four-stage random sampling technique. An interviewer-administered questionnaire which was pre-tested was used to collect data on socio-demographic and CF mode of consumption. Dried CF larvae from Nigeria, Ghana, and Burkina Faso were purchased from Iseyin, Bodija market, Bobo-Diollaso respectively.Four vegetable soups:(vegetable+larvae,vegetable(control), Vegetable+egusi+larvae and vegetable+egusi (control)) were prepared using traditional method.Standard methods were used to analyze the chemical composition of the three larvae and four vegetable soups. Toxicological and nutrient bioavailability evaluations were done using Wistar rats following OECD guidelines, while the microbial assessment was done using the pour-plate method. Vegetable soups acceptability rating was conducted among 30 untrained panellists using a 9-point hedonic scale. Descriptive statistics, independent sample t-test and ANOVA were used to analyse data at $\alpha_{0.05}$.

Respondents' age was 46.3±16.4 years and 11.8% had tertiary education. Most respondents (99.8%) were aware of and had consumed CF larvae, 35.4% consumed it as roasted/dried, 35.9% as boiled/fried and 74.9% as a condiment in soups. One hundred grammes of dried CF sample contained 3.8±0.35g moisture, 53.2±1.10g protein, 16.9±0.23g fat, 2.6±0.09g ash, 453.3±0.08kcal energy, 8.9±0.02mg iron, 633.3±0.01mg potassium, 270.6±0.01mg calcium, and 1.4±0.03mg zinc. There was no significant difference in haematological and serum chemistry values of rats fed with control and CF-supplemented diets at 20% inclusion level, but significant

weight gain was noticed in rats fed with CF-supplemented feeds (37.3g) compared to the control (29.5g), with an increase in alkaline phosphatase at 80% inclusion level indicating a negative effect of the rat liver. Serum protein (5.1±0.14g/dL), ferritin (26.0±0.55mg/dL), vitamin A (40.2±0.99µg/dL) and zinc (5.6±0.44mg/dL) concentrations of the experimental diets were significantly higher than that of basal diet at 4.1±0.14g/dL, 22.4±1.37mg/dL, 31.1±0.48µg/dL and 3.1±0.49mg/dL respectively. Larvae-enriched vegetable soups had higher nutrient content than plain vegetable soups and were more suitable in terms of aroma, colour, taste and texture. Total heterotrophic microflora counts (25.3-39.2×10^5) of dried CF were within the acceptable limit after 180 days of storage.

Cirina forda larva is rich in macro and micro-nutrients which are bioavailable. Its consumption posed no health hazard in rats, hence, its consumption is recommended for human, as it has the potential to reduce protein-energy malnutrition and micronutrients deficiencies among consumers.

Keywords:*Cirina forda*larva, Nutrient bioavailability, Larva toxicity, *Cirina forda* consumption.

Word counts: 500

DEDICATION

This intellectual effort is devoted to God for HIS grace over me and my household.

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CI:	Consumption Index
ECI:	Efficiency of conversion of Ingested feed
FAO:	Food and Agriculture Organization
GR:	Growth rate
IFAD:	International Fund for agricultural Development
IITA:	International Institute of Tropical Agriculture
MD:	Micronutrient Deficiency
NNHS:	National Nutrition and Health Survey
PEM:	Protein-Energy Malnutrition
PER:	Protein Energy Requirement
RDI:	Recommended Dietary Intake
WFP:	World Food Programme
PUFA:	Polyunsaturated Fatty Acids
SAM:	Severe Acute Malnutrition
UNICEF:	United Nation International Children's Emergency Fund
AOAC:	Association of Official Analytical Chemists
SDG:	Sustainable Development Goals
OECD:	Organization for Economic Co-operation and Development guideline for testing of Chemicals
ALT:	Alanine Aminotransferance
AST:	Aspartate Aminotransferance
PCV:	Packed Cell Volume
PER:	Protein Efficiency Ratio

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Entomophagy is a word often used to describe the consumption of insect by man. The practice is culturally universal but varies with locality, kind of insect and folkloric setting of consumption (Johnson, 2010). Insects are customarily consumed in most cultures given the significant role in human nutrition. Aside from being reared for economic purposes, they possess high nutritional value and are quite famous in numerous cultures around the globe particularly as special delicacies and replacement diets in periods of food dearth. Their utility for emergency sustenance during starvation is rare, but are a regular part of diets during the year or in seasons of commonness as delicacies such as nibbles or condiment in soups (Banjo*et al.,* 2006; Alamu*et al.,*2013; Adeoye*et al.,* 2014).

The history of human nutrition across the world is incomplete without stressing the vitality of insect consumption given the species in thousands that are being part of the human diet. Grasshoppers, insects' caterpillars, beetle grubs,termites, crickets, wasps, bees,ant-brood (pupae and larvae) cum winged ants, cicadas, and a lot of insects most especially aquatic, are some of the important edible insect (Adeoye *et al.,* 2014) that forms part of dietary components in several cultures with meaningful contributions to protein, fats, vitamins and minerals requirements in diets (Womeni *et al.,* 2009; Amadi and Kiin-Kabari, 2016; Payne *et al.,*2016).Iombor *et al.,* (2017) stated thatedible insects contain a substantial sum (40-75%) of quality and highly digestible proteins with valuable fatty and amino acids profile analogous to the regular livestock and fish. They comprise an amino acid that is lacking in the regimes of grain-dependent populations and also a huge reserves of lysine (DeFoliart, 1992); making them a promising alternative in the alleviation and management of malnutrition in Nigeria.

In Malawi, Zambia and Tanzania, eating dried-caterpillar flour is a viable strategy for fighting malnutrition among children, women who are pregnant, nursing mothers and patients who are suffering fromanaemia because of its richness in protein, minerals especially calcium and iron (Igbabul*et al.,* 2014). Besides, insects are a vital part of the culture (rituals) with useful medicinal value all through ages.Edible insects are important, noble and preferred reserves of protein, vitamins, minerals and fats (Adeoye *et al.,* 2014, Durst and Shono, 2010; Alamu*et al.,*2013) analogous to that of meat and fish and in some cases have a greater protein, fat and energy stores (Alamu *et al.,* 2013). However, the taste and nutritional worth differ with species, metamorphic stage and habitat (Agbidye *et al.,* 2009).Consumption of edible insects is a traditional practice in many African (Van Huis, 2003), Asian (Yen, 2015) and Latin American (Costa-Neto, 2016) communities. More than 2000 species of insects are suitable for human consumption worldwide (Jongema, 2017). DeFoliart (2002) described many edible insects of various species as vital kinds of stuff of trade in local markets across Africa and sub-tropical areas of the world.

The major consumed insects arecaterpillars (Lepidoptera)–18%, flies (Diptera)–2%, ants and wasps (Hymenoptera)–14%,termites (Isoptera)–3%,crickets, locusts and grasshoppers, (Orthoptera)–13%,dragonflies (Odonata)–3%, plant-hoppers, leafhoppers, scale insects, cicadas and true bugs (Hemiptera)–10%, bees, beetles (Coleoptera)–31%, and others –5% (FAO, 2013).Among these, is a Pallid emperor moth, *Cirina forda* Westwood larva from order Lepidoptera, an edible insect often commonly under-reported. The larvae look similar to silkworm caterpillars, do not spin cocoons, but burrows into the ground to the foot of the host plant to pupate. It is usually referred to as 'Konniwole' in Southwestern Nigeria among Yoruba natives; 'Kanni' meaning*Cirina forda* while 'wole' infers to enter the soil (Osasona and Olaofe, 2010). Some cultural settings in the Southwestern called it 'Monimoni'. The *Cirina forda* larvadefoliatesShea butter leaf before entering the soil; and is collected during raining season (Vantomme*et al.,*2004).*Cirina forda* larva is extensively eaten in Nigeria mostly in the North and South-West (Ogunleye, 2006; Agbidye *et al.,* 2009).Proximate investigation of *Cirina forda* larvae showed protein and energy value of 64.49% and 479.61mg/g respectively on a dry weight base (Ande, 2002). Larvae are usually eaten raw, roasted or dried as snacks or an essential partof vegetable soups which serve as an ingredient (Omotosho, 2006).

1.2 Statement of the problem

Undernutrition is a result of poor nutrition with inadequate access to nourishing foods, thereby impairing the nutritional needs for protein, energy and micronutrients. Approximately, 780 million people out of 795 million global malnourished population originated from developing countries (FAO, IFAD, WFP, 2015). Protein-Energy Malnutrition, PEM as well as micro-nutrient deficiencies are the main public health concerns in average income settings (partly as a result of sky-rocketing food costs particularly those of animal origin – Oibiokpa *et al.,* 2018). However, the potential contribution of edible insects to food security in continents such as Africa and Asia is under threat. A decrease in the prevalence of traditional practices of entomophagy has been reported in communities indeveloping countries where insect consumption used to be common (Dube *et al.,* 2013; Meyer-Rochow and Chakravorty, 2013; Obopile and Seeletso, 2013; Riggi *et al.,* 2016; Yen, 2009).Reasons for this decrease include the adoption of Western foods (Dube *et al.,* 2013; Looy *et al.,* 2014; Mlcek *et al.,* 2014; Obopile and Seeletso, 2013; Yen, 2009) and decreased knowledge of preparation practices (Riggi *et al.,* 2016). Other reported reasons include unavailability of edible insects (Looy *et al.,* 2014), uncontrolled harvesting (Ramos-Elorduy, 2006), and loss of habitats leading to the extinction of some species (Dube *et al.,* 2013; Meyer-Rochow and Chakravorty, 2013). In developing countries, especially in urban areas and younger populations, there is a tendency to abandon the practice of entomophagy due to the westernisation of traditional diets (van Huis and Vantomme, 2014; Vantomme, 2015). Also, there is a common belief that traditional foods, like edible insects, are considered to be primitive and are not accepted by Western communities. This thinking leads to the unwillingness of people to share experiences of these foods (Looy *et al.,* 2014).

1.3 Justification for the study

The vitality of insect consumption as a preferred and essential food source of proteins, fats, minerals and vitamins cannot be underscored in several developing societies and different cultures of the world (Durst and Shono, 2010). They exert nutritional potential comparable to that of meat and fish (De Foliart, 1992) and are habitually welcomed as a substitutesource of protein to animal meat (Sponheimer, de Ruiter and Lee-Thorp, 2005).

Multiple studies stress the importance of documenting traditional knowledge of edible insects to restore and promote entomophagy (Riggi *et al.,* 2016; van Huis, 2015; Yen, 2009) and to

disseminate information to new consumers, especially in urban areas (Gahukar, 2011).*Cirinaforda* (Westwood) larvae, as well as other edible insects, had been reported to be very nutritious, widely acceptable and commonly eaten in Nigeria. Its supply is almost a gift by nature, and it could serve as a reliable reserve of protein, calcium, iron and zinc with a promising potential in reducing micronutrient malnutrition. However, there are gaps in information on the consumption pattern of *Cirina forda* larvae in southwestern Nigeria, with no evident study on its nutrient bioavailability in humans though it is reportedly rich in protein, iron, zinc, and calcium. Some insects have been found to contain some pharmacologicallypotent substances (known as toxins) for self-defence and other reasons. Thus, the study of the toxicity of *Cirina forda* larvae to ascertain whether its consumption is safe or could portend any significant risk to the health and well-being of its consumers cannot be overlooked.

1.4 Research questions

- How popular and acceptable is *Cirina forda* larva in the study area?
- Does the consumption of *Cirina forda* larva pose health risks to the consumers?
- What is the proximate nutrient composition of *Cirina forda* larva?
- What are the important minerals and vitamins present in *Cirina forda*?
- What are anti-nutrientcomponent in *Cirina forda* larva?
- How bio-available are the nutrients in the *Cirina forda* larva when consumed?
- Is there any reported case(s) of poisoning upon its consumption in the study area?
- How do people prefer the consumption of *Cirina forda* in vegetable soups?
- How long can *Cirina forda* larva be preserved or stored at different storage conditions?

1.5 Objectives of the study

The general objective of the study was to examine the consumption pattern, nutrient composition, nutrient bioavailability, toxicology and consumer acceptability of *Cirina forda* (Westwood) larva-enriched vegetable soups.

1.6 Specific objectives

Specific objectives of this study were to:

1. Assess the consumption pattern and knowledge of nutritional benefits of *Cirina forda*larva in the selectedlocations
2. Determine and compare the nutrients and anti-nutrients composition of *Cirina forda*larvae obtained from the selected locations
3. Carry out toxicological studies on *Cirina forda* larva using rat models.
4. Evaluate the nutrient bioavailability of *Cirina forda* larvae using experimental rats.
5. Conduct sensory evaluation and determine nutrient composition of *Cirina forda* larvaeenriched vegetable soups
6. Assess the effect of storage conditions on the shelf-life of dried *Cirina forda* larva

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition

Malnutrition, in all its forms, includes undernutrition (wasting, stunting, underweight), inadequate vitamins or minerals, overweight, obesity, and resulting in diet-related non-communicable diseases. According to WHO, 2020 about 1.9 billion adults are overweight or obese, while 462 million are underweight. Approximately 47 million children under 5 years of age are wasted, 14.3 million are severely wasted and 144 million are stunted, while 38.3 million are overweight or obese.Around 45% of deaths among children under 5 years of age are linked to undernutrition. These mostly occur in low- and middle-income countries. At the same time, in these same countries, rates of childhood overweight and obesity are rising.The developmental, economic, social, and medical impacts of the global burden of malnutrition are serious and lasting, for individuals and their families, for communities and countries.Malnutrition refers to the cellular disproportion between the nutrients supply (including energy) and the physiological demand to promote maintenance, growth and precise tasks (WHO, 2019).

Nigeria is rated as the second-highest of short children andthe frequency rate of 32 per cent of children who are below five years old (UNICEF, 2019). In Nigeria, approximately 2 million children suffer from extreme severe malnutrition (SAM) and, sadly, only 2 out of 10 affected children are presently seeking care. Acute malnutrition also affects 7% of young adult women who have attained the age of childbearing.Malnutrition is a wide-ranging term that implies both under-nutrition and over-nutrition. In Africa, malnutrition accounts for the highestburden of mortality and morbidity, stunted growth and impaired cognitive growth in under-five children (Iombor *et al.,* 2017).It represents one in every three deaths of the overall eight million mortality in under-five children annually (Black *et al.,* 2010; Rajaratnam, 2010).

Stunting and wasting are the two types of malnutrition's that affect most children in the Northern part of Nigeria. This disturbing high rate of malnutrition presents serious challenges for the country in terms of public health and growth. Stunting is related to impaired cognitive development, decreased educational success in children and low adult productivity. This leads to losses in the economy, which could account for an average of 11% of the Gross Domestic Product (GDP) of Nigeria.

Undernutrition is a complex medical illness characterized by diet-deficient of most essential nutrients. Over 90% of the about 795 million people undernourished globally are from developing regions (FAO, IFAD and WFP, 2015). One in every five under-five children are suffering from protein-energy malnutrition, PEM and approximately average of all mortality under-five children are attributable to undernutrition (FAO, 2015). Similarly, 32%, 19.9% and 7% of under-five are stunted, underweight, and wasted respectively (NNHS, 2018). PEM and Micronutrient deficiency, MD is a key importancepublic health in the developing countries mostly attributed to poor access to adequate nutritious foods (due to high food prices especially those of animal origin) (Oibiokpa *et al.,* 2018), inadequate food intake and poor feeding practices (Adepoju and Ajayi, 2016).

Toprevent malnutrition, UNICEFadvocated by encouraging sensitization (education) and guidance to mothers and caregivers about proper feeding to children and caregivers, and by supplying children and pregnant women with adequately free micronutrient supplements.

However, the high nutritive values of edible insects present them as a possibly healthy reserve of fats, proteins (13–77% of dry matter), vitamins, fibre and minerals (Xiaoming *et al.,* 2010), thereby making them a suitable alternativein combating malnutrition particularly because traditionally-prepared edible insects larvae powder have been used as complementary foods and food supplements in infant feeding.

2.2 Entomophagy

Entomophagy means the consumption of insect by humans. It is a term derived from the Greek word entomos (insect) and phagein (to eat), the practice is a well-established although a diminishing custom of many parts of the world (Sutton, 1998; DeFoliart, 1999). Entomophagy is culturally universal but varies from locations, insect type and ethnic setting (Johnson, 2010).

Insect consumption is part of a population's cultural heritage, and that 1391 insect species are eaten worldwide, of which 524 are eaten in 34 countries Africa representing 38% of all species consumed globally, she maintained that Mexico consumes 348 species which is the highest number recorded for a single country (Ramos-Elorduy, 1997). Jongema (2012) holds a contrary opinion and posits that about 1900 species of insects are eaten worldwide, mainly in developing

countries. Bodenheimer (1951) maintained that the people of Madagascar love to eat fried grasshoppers, Bahuchet (1990) posits that caterpillars are a very important food item for the Pigmies, Kitsa (1998) wrote that in a certain city in Southwest DR Congo 28% of its inhabitants eat insects, Roulon-Doko (1998) maintained that about 15% of the meat diet of the Gbaya people in Central African republic consist of insects. Adriaens (1951) reported that between 1954 and 1958 close to 300 tonnes of dried caterpillars were consumed yearly in the Kango district of DR Congo. Furthermore, some tribes in Colombia, Venezuela, and South Africa preferred certain insects to fresh meat (Quin, 1959). Though insects are seen as food for the poor and backward by some, they are sought after and served in the best restaurants in most countries of Asia, Australia, and Europe (Ramos-Elorduy, 2009; Yen, *et al.,* 2012). Robert (1989) indicated that a 10% increase in the world supply of animal protein through the mass production of insects for food can to a large extent reduce if not eliminate the malnutrition problems of the world and also decrease the pressure on conventional protein sources.

Edible insects have been used as foods across different populations and cultures for several years worldwide (Adeoti *et al.,* 2013) and they form part of the customary food of over two billion people globally (Jideani and Netshiheni, 2017) with vital roles in human nutrition and also reared for profitable purposes given their high nutrient quality. They deliver a host of ecological amenities critical for human survival. Well over 1900 species of insects have been reported as foods (Jongema, 2014). Flies (Diptera, 2%), termites (Isoptera, 3%), dragonflies (Odonata, 3%), cicadas, leafhoppers, plant-hoppers, scale insects and true bugs (Hemiptera, 10%), grasshoppers, locusts and crickets (Orthoptera, 13%), bees, wasps and ants (Hymenoptera, 14%), caterpillars (Lepidoptera, 18%), beetles (Coleoptera, 31%), and others (5%)are the most frequently consumed forms of insects across the world.

However, insect consumption is becoming an uncommon phenomenon in developed countries but residues a widespread practice in some developing provinces of Central and South America, Africa and Asia (Adeoti *et al.,* 2013). They are favourite instead of meat in some localities due to their low Poly Unsaturated Fatty Acid, PUFA contents. Presently, edible insect consumption is gaining more popularity for their nutrient and environmental comparative advantage over the production of meat (Van Huis *et al.,*2013). Their diminutive life span, low requirements for space, effectual rates of nutrient conversion and lesser production of greenhouse gas makes them

a promising exceptional substitute for meat (Van Huis and Vantomme, 2014). Reports from Nigeria have demonstrated the direct or indirect experiences of entomophagy among Nigerians with widespread practice in rural settings than urbanized areas (Igwe *et al.,* 2011).

According to Olaofe *et al.,*(1998), one of the possible ways to counter protein-energy malnutrition in Nigeria is to promote the utilisation of lesser-known and cheaper sources of animal proteins such as those from insects. Scholars have written enormously on entomophagy among several tribes in Nigeria, however, most writers maintained that the practice is more prevalent among rural populations than urban population (Fasoranti and Ajiboye, 1993). There is a significant trade-in and consumption of edible insects among some ethnic groups in Nigeria. Akingbohungbe, (1992) and Banjo *et al.,* (2006) maintained that grubs of palm weevil are fried and eaten in several parts of Western Nigeria, Edo, and Delta state. Also, Adamolekun (1993) reported that larvae of *A. venata* are eaten among the people of Southwestern Nigeria. The larvae of the Saturnid Caterpillar (*Cirina forda*) known locally as 'Kanni'is widely eaten among the people of Kwara state. It is believed that this caterpillar is the most widely marketed edible insect in Nigeria and sells for about twice the price of beef and is used for vegetable soup. Other widely marketed insects in Nigeria include termites and dragonflies (Fasoranti and Ajiboye, 1993). The Gwari people of Niger state eat the larvae of dung beetle (*Aphodius rufipes*) and to them are a delicacy and serves as an alternative source of protein (Paiko, 2012).

Ene, (1963) maintained that many insects or their products were used as food in some parts of Nigeria and to a large extent eaten as titbits or exclusively by children. 'Ordinarily, insects are not used as emergency food during the shortage, but are included as planned part of the diet throughout the year or when seasonably available' (Banjo *et al.,* 2006). Banjo *et al.,* (2006) further gave the following as the list of commonly eaten insects in South-western Nigeria; *Macrotermes bellicosus,* Brachytrypes spp., *Oryctes boas*, *Rhynchophorus phoenicis, Apis mellifera* (oyin), and *Cirina forda* (ekuku).The most abundant, most preferred, and most consumed insect by the people of Benue State, Nigeria is *Macrotermes natalensis* followed by *Brachytrupes membranaceus* and then *Cirina forda* (Agbidye *et al.,* 2009). In Borno state, northern Nigeria, *Zonocerus variegatus* is widely eaten and seen as a delicacy; the grasshoppers are readily displayed in the market and sold like meat (Solomon *et al.,* 2008). With the'rising global population and limited pasture lands, production of animal protein is becoming ever

unsustainable and one of the possible ways to overcome this problem is to adopt the entomophagy practice' (Abassi and Abassi, 2011). More so, whether insects are eaten or do not depend on the low nutritional value of insects, but often such is tied to customs, preference, or and prohibitions (van Huis, 2003), and in some societies, it is just a taboo (Weiss and Mann, 1985; McElroy and Townsend, 1989). Awareness of entomophagy and its advantages are required as part of the solution to protein-deficient malnutrition in the developing countries, Nigeria inclusive.

2.3 Factors militating against entomophagy

Insect consumption is very beneficial to humans, but certain factors appear to hinder its acceptability especially among people who can afford to buy meat, fish or egg and other high-priced animal proteins. These factors include:

Seasonality: Seasonal availability of the vast majority of edible insects has a deleterious impact on entomophagy because their availability is reliant on weather conditions and seasonal climates.

Education and Modernization: In the report of Ene (1963), edible insects as too murky or repulsive to be considered as food given they have been reported as pathogens of disease organisms. Modernization and shifting attitudes are negatively affecting entomological practices given the perception that it is outdated. The study posited many educated West African populace are perhaps ignorant or unwilling to admit the actuality of some traditional customs including insect consumption and are in any case is a reason for their under-utilization. In most climes, entomophagy is perceived to be is related to poverty, famine, non-availability of meats and fishes and among others (Owen, 1973).

Customs (Taboos) and Religions: Taboos and religions are parts of important factors influencing edible insects consumption (Fasonranti and Ajiboye, 1993). For example, children are not allowed to feed on thetermite's queen for several reasons unrelated to safety and health. Because children are a major part of the farmlands and farming in African settings, elders accept as true that allowing children to eat the queens of termites makes them cherish and unnecessarily spend much time searching the insect thereby reducing their productivity on the farm fields. Similarly, in eating palm weevil larvae, children are discouraged given its good sense of taste

may perhaps preoccupy them with clear-felling palm trees to offer additional breeding spots and harvesting huge larvae and primary palm products; oil, kernels and wine stand at risk of deprivation. Furthermore, the "Ire" the Yoruba clique community in Nigeria avoid eating crickets, being predominantly blacksmiths worshipping "god of Iron – Ogun" that does not accept non-red bloodied animals (Fasoranti and Ajiboye, 1993)

2.4 Sources of edible insects in Nigeria

Consumption of insect is a common phenomenon throughout every part of the world (Banjo *et al.,* 2006).Previously, native insect consumers were typically domiciled in the rural parts of Nigeria. While, some edible insects are obtainable in all seasons of the year and are usually collected in the forest (Fasoranti and Ajiboye, 1993), others are short-lived by season because of climatic conditions (Adeduntan and Bada, 2004; Ashiru, 1988). Before now, edible insects such as termites, grasshopper, cricket are widely available and often collected for home consumption by the people in the rural (Agbidye *et al.,* 2009; Muyay, 1981). However, insect availability has significantly decreased in terms of quantity and species due to an upsurge in the quest for edible insects and a decrease in insect habitats arising from modernization or industrialization (Agbidye *et al.,* 2009).

2.5 Harvesting, processing and consumption style of edible insects

Edible insects are usually consumed at the different phases of their metamorphic lives. (Chavunduka, 1975). For instance, while grubs are consumed in larval or pupae stages, grasshoppers, crickets and termites are consumed during adulthood (Banjo *et al.,* 2006). Due to the seasonal availability of many insects, they are collected or harvested and preserved for consumption. They are cooked, dried or roasted and served in countless methods and commercially dispensed at roadside food markets or stalls and the markets in villages, towns and metropolises of Nigeria (Agbidye, *et al.,* 2009). Nigerians from undeveloped settings have applied age-long indigenous knowledge in processing edible insect for consumption.

Table 2.1: Sources and seasonal availability of commonly consumed insects

Scientific name	Host Plant	Seasonal Availability

Oryctes boas	Coconut tree, oil palm and raphia palm	During rainseason
Rhynchophorus Phoenicis	Oil palm and raphia palm	By the completion of the wetseason (September-October)
Cirina forda	*Vitellaria paradoxa,* formerly calledButyrospermum spp	June-August
Zonocerus Variegates	*Manihot esculenta,* green plants.	Commonly seen by the dry season (November-April).
Bunaea alcinoe	*Parkia biglobosa, Prosopis*spp.	Majorly between July-August
Macrotermes natalensis	Tree species	During rain season
Brachytrupes membranaceus	Soil and underground tunnel of tree forest	Throughout the year
Anaphe venata	*Triplochiton scleroxylon*	Rain Period (July-September)
Analepte strifasciata	Oil palm, Raphia palm	End of the rainy season (September-October)

Source: Banjo*et al.,*(2006)

Table 2.2: Characteristic of harvesting, processing and local cooking style

Insect	Local name	Harvesting	Processing
Termite: Macrotermes spp.	Aku (Ibo), Esunsun(Yoruba)	Electric light attracts termites, hence, they are trapped in a bowl with water placed near the light source. In the villages, they are caught at the termite mound.	Termites are roasted in a pot, wings broke off
Rhinoceros beetles: *Oryctes boas*	Ogongo(Yoruba)	Through handpicking	Collected larvae are washed, fried and condiments such as pepper, salt, onion or spices added
African Palm Weevil: *Rhynchophorus* *Phoenicis*	Ipe (Yoruba) Ebe (Ibo)	Through handpicking from dead raphia or oil palm	The abdomen that contains gut is removed, larvae washed, fried and condiments added to spice the delicacy
Pallid emperor moth or Shea defoliator: *Cirina forda*	Kanni or Monimoni (Yoruba)	From the leaves on the Shea-butter trees, orin and/or on the soil around the tree bases is where the larvae are collected.	The larvae are starved for one or two days to remove the abdominal contents,boiled for two hours, removed the hair, washed, then sun-dried on mats. The dried can be roasted before cooked in stew or with vegetable soup
Grasshopper: *Zonocerus variegates*	Tata (Yoruba), Abuzu (Ibo)	They are either hand-pickedor the use of sweep net on the field/farm.	Grasshoppers are starved to remove gut, wings, and legs.Then roasted and added Condiments to garnish to taste
Cricket: *Brachytrupes* *Membranaceus*	Ire (Yoruba)	From soil tunnels which they build	The crickets are removed, roasted, before eating or can be cooked with stew or with vegetable in soups.
Silkworm: *Anaphe* *Venata*	Ekuku (Yoruba)		For one or two days, the larvae are starvedto remove the gut substance,sun-dried and boiled on mats. Stew with vegetables could be cookedand adding a needed condiment

Source: Banjo*et al.,*(2006)

2.6 Nutritional benefit of edible insects

The nutritional importance ofinsects differs by species, metamorphic stage, habitat, diet exposure, and method of preparation and/or processing. Edible insects interlink biodiversity, conservation, and nutrition in an unprecedented manner than other conventional food sources because they contain proteins, fats, and carbohydrates uniquely greater than that of meat (beef) or fish, as well as higher energy worth than maize, soy-beans, beef, lentils, and fish, etc. (FAO, 2013). Besides, Nkouka (1987) has reported the similarity in the roles and functions of edible insects and meat in the human body.Edible insects are better sources of nutrients which are fats, protein,vitamins and mineral with large storage of lysine – an amino acid deficient in most diets of grain-dependent populations (DeFoliart, 1992). They have higher food conversion competence and reproduction rate than many traditional types of meat and beef respectively. Thus, protein production for human consumption through edible insects is not only promising but also cost-effective compared to animal protein (Capinera, 2004), making it an ecologicallyviable alternative to vertebrate meat.

Similarly, the protein potential of insects that are edibles is not only high when compared to fish and meat, but is easily assimilated in the human body just as plant protein (Bukken, 1997). Also, their energy contents are on average in comparison to that of beef (basis of fresh weight) but not with pork because of its exceptional reserves of high-fat.The nutritional benefit of insects is often overlooked, and some do not even know that insects are of any nutritional importance. Insects are good sources of essential nutrients that could help alleviate the nutritional problems among the populations that consume them (Ekpo, 2011) Documentation of the nutritional importance of insects to the human diet is sparse (Roos, 2012). The prejudice against eating insects is not justified from the nutritional point of view (van Huis, 2003). According to Meyer- Rochow (2010), Bodenheimer (1897-1959) was the first scholar to write about the nutritional importance of insect in his book ‘Insects as human food’ in 1951. Insects are a source of protein for the improvement of the human diet; they are an important source of protein for people who are malnourished. Some insects contain more protein than meat (Johnson, 2010). Bergeron *et al.,* (1988) maintain that the crude protein of three aquatic insects eaten around Lake Victoria Uganda to be 67%. Insects contain a high amount of crude protein. In g/100g dry weight caterpillars contain 50-60, palm weevil larvae 23-36, Orthoptera 41-91, ants 7-25, and termites

35-65 (Bukkens,1997). According to Santos *et al.,* (1976) 100 grams of a caterpillar would provide 76% of an individual's daily protein requirement and more than 100% of the daily requirement for many of the vitamins and minerals. For example, the mean values in protein percentages of insects in their mature stages, and adult stages are 36, and 38 respectively, and some are as high as 72 and 69 (wet weight). Tsvangirayi (2013) posits that the Emperor moth (Mopane worm) contains three times the amount of protein as beef. The crude protein value of Rhynchophorus sp. is as high as 71.6%. In a study of 94 edible insects by Ramos- Elorduy and Pino Moreno (1990) they found out that 50% of the insects have higher caloric values than soybeans, 63% were superior to beef, and 70% were better than fish and beans. Many insects are low in cholesterol and fat (Srivastava *et al.,* 2009), they are herbivores and have clean eating habits which makes them cleaner than chicken, pigs, and many other conventional protein sources the grasshopper is one of the cleanest animals (Abbasi and Abassi, 2011). Most often than not, most insect consumed are harvested from the wild, or come from the wild and are gathered for food, hence, they are mostly free from pesticide and other chemical contaminants which abound in places where the conventional source of protein are found (Durst and Shono, 2010). When insects feed on the vegetation they can transform phytomass into zoomass much more efficiently than conventional livestock; more than 10 times more plant nutrients are needed to produce one kilogram of meat than one kilogram of insect zoomass (Nakagaki and DeFoliart, 1991; Taylor, 1979). Furthermore, when an insect is generally eaten by most social insects especially those of them that are edible would be seen not as a pest but as the source of much-needed protein. The use of pesticide and other chemicals which harm health and the environment would be brought to its barest minimum. More so, some locust species despised and considered dangerous to crops in tropical Africa are seen as delicacies by people of other land and culture (Owen, 1973).The edible insects' nutrient composition of 236 (based on the dry matter) was reported by Rumpold and Schlüter (2013) with noteworthy disparities in reported data but satisfactory quantities of energy, proteins and amino acids that are required by a human. Besides, great profiles of monounsaturated and/or PUFA, and micronutrients (such as iron, magnesium,copper, manganese, selenium, phosphorous, pantothenic acid, zinc, riboflavin, and biotin (in some cases, folic acid) were as well reported.

In Nigeria, several reports (Ashiru (1988), Fasoranti and Ajiboye (1993), Ekpo, Udoh, Akpan. (2010), Banjo, Lawaland Songonuga, (2006), Ifie and Emeruwa (2011), Agbidye, Ofuya & Akindele (2009)and Adepoju and Ajayi (2016)) studied the nutrient composition of some comestible insects ranging from Orthoptera, Coleoptera and Lepidoptera to Hymenoptera and Isoptera species revealing protein and energy contents (Ajayi and Adedire, 2007). For example, Agbidye *et al.,*(2009)reported that*Cirina forda*contains 74.35%protein on a dry weight basis. Also, Banjo *et al.,*(2006) demonstrated the crude fibre profiles in terms of g/100g dry weight of *Macrotermes natalensis*, *Oryctes boas*, Anaphe spp. and *Rhynchophorus phoenicis* was 7.85%, 3.40%, 1.68 - 3.10% and 2.82 % respectively.

Generally, edible insect possesses large stores of calcium and phosphorus. For example, 61.28mg/100g and 136.4mg/100g of calcium and phosphorus respectively were reported in *Analepte strifasciata*. Also, iron, phosphorus and vitamin A stores ofMacrotermes spp. were 27-29mg/100g, 136mg/100g and 2.56-2.89µg/100g respectively in addition to 85mg/100g iron store for *Oryctes monoceros* (Banjo *et al.,* 2006; Ifie and Emeruwa, 2011). Also, 50g consumption of dried caterpillars has been reported by Malaisse (1997) to secure recommended daily allowance (RDA) for riboflavin (B2), pantothenic acid (B5) and 30% of the requirement of niacin (B3) in humans.

2.7 Food safety of edible insects in Nigeria

Globally, the vitality of insects that are edible as traditional foods in many ethe in the world cannot be underscored in the science of human nutrition. Concerted efforts geared towards promoting the use of edible insects as a vitalnutritional food source is not only necessary but timely in mitigating the risk of malnutrition given the Nigerian economy is currently experiencing a recession. Viable and evidence-based researches on the noxiousness and safety of food of edible insects are necessary to alleviate consumers' apprehensions. For example, Ashiru (1988) reported Anaphe larvae is a good source of fat with 6.113 kcal/g calories, but recent studies implicated the edibility of this larva as a risk factor for the seasonal ataxic syndrome. However, the generalization of these findings is quite limited given the absence of animal model trials and clinical trials on this subject.

Though edible insects appear less difficult than omnivores given their generally herbivorous background, pesticide use is a potential threat debarring their suitability for human consumption because certain reports have established the bio-deposition of herbicides (arising from the consumption of edible plants) in the edible insect. A case in point is the grasshopper *C. naeruginosus*(unicolour) and *Zonocerus variegates*were collected for auction as condiment food in Akoko, Ondo State Nigeria, but later found unsuitable for consumption given the possible contamination with organo-phosphorus pesticides.

2.8 Others Importance of insects

2.8.1 Environmental benefits

Given the cold-bloodedness of insects, they exert high feed conversion competence. Averagely, insects are capable of converting 2 kg feed into1kg mass of insect, while cattle need 8kg of feed to manufacture 31kg of meat. Despite this, feed-to-meat exchange rates differ extensively relying on the group and class of animal and the methods of production. Also, greenhouse gas production by nearly all insects is plausibly lesser than that of usual farm animals. For example, the production of greenhouse gases in pigs is 10–100times/kg more than in mealworms. The mealworm can degrade bio-waste and transform it into high-quality protein applicable for agricultural feed. Besides, they are drought-resistant and less land-dependent than conservative animal farming.

2.8.2 Health benefits

The metamorphic stage of insect largely influences their nutritional content. However, it is extensively referred to that insect are vital repositories of protein and nutrients that are of highquality compared to fish and meat. They serve as a significant food supplement in alleviating the burden of undernourishment among children given their high fatty acids stores, rich fibre and micronutrient (Se, Cu, Mg, P, Mn, Znand Fe) reserves. Also, they pose an insignificant danger of zoonotic diseases (such as; H1N1 – bird flu, BSE – mad cow disease) transmission.

2.8.3 Livelihood and social benefits

Rearing and gathering insects for marketable purposes offer vital means of livelihood to humans. They can be easily harvested, processed and outsourced (with minimal scientific knowledge, capital and equipment) in the wild particularly in the rural areas to provide means of livelihoods and cash income in-flow to women, vulnerable children as well as men.

It can also offer free enterprise privileges too for high and low economies. Edible insects can be transformed into pastries or meals with their protein's stores haul out for nutritional rationale, for example, the value and importance of honey and silk have been reported in the literature. Almost one and a half million tonnes of profitable honey are provided by bees annually (FAO, 2009b), silkworms are responsible for not less than ninety thousand tonnes of silk production annually (Yong-woo, 1999), Carmine (a red originating from scale insects of order Hemiptera), is used in the colouring of foods, fabrics and drugs and Resilin; an elastic-like protein that allows insects to leap, has been applied in repairing the arteries due to its tensile and flexible characteristics (Elvin *et al.,* 2005).The use of bee products and therapy by Maggot; propolis, honey, venom and royal jelly in the management of trauma, wounds and burns are other applications of insect therapy in medical sciences (van Huis, 2003a).

2.9 *Cirina forda* larva distribution in Nigeria and Africa

The shea-defoliator or pallid emperor moth,*Cirina forda*is an insect pest of *Vitellaria paradoxa* formerly called *Butyrospernum paradoxum* – the shea-butter tree. The leaves of the shea tree are what the larvae feed on. An English entomologist named John Obadiah Westwood in 1849 was the first to describe the species. *Cirina forda* larvais the majorextensively consumed insects in Southwest Nigeria (Fashoranti and Ajiboye, 1993). *Cirina forda* is also important for commercial purpose in many states; Benue, Kaduna, Kogi Kwara Niger, and Oyo states as a mean of commerce. The larvae are transformed into the dried-out form and widely sold for consumption as part of vegetable soups and snacks with carbohydrate food in Nigeria (Fasoranti and Ajiboye, 1993, Anthonio and Isom, 1992). The larvae can also be boiled excising the gut and sun-dried before sales. *Cirina forda* larvae are widely consumed in Nigeria, Burkina Faso, Cote d'Ivoire, Togo, and Ghana (West Africa); Central Africa (Democratic Republic of Congo-Kinshasha) and Southern Africa (South Africa, and Zimbabwe)(Kelemu *et al.,* 2015).

2.9.1 Classification of *Cirina forda*larva

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Saturniidae
Genus: Cirina spp.
Species: *Cirina forda*

Plate 2.1: *Cirina forda* larva defoliating sheabutter, *Vitellaria paradoxa* leaves

Source**:**(Rik, 2017)

Plate 2.2: Dry processed *Cirina forda* (Westwood) larvae

Source: Picture of *Cirina forda* larvae taken as bought from the market

2.10 Leafy vegetables

The word vegetable refers to the tender consumable or edible sprout, leaves, fruits and roots of florae consumed in whole or part, cooked or raw as a supplement to carbohydratesfoods and as an alternative to meat (Olasanta, 2015). Leafy vegetables (also known aspotherbs, greens,salad greens,vegetable greens, or leafy greens), are florae greeneries consumed as a vegetable, occasionally complemented by tender petioles and stems. They differ in origin and variety but share a common heritage of nutrition worth and culinary methods.

Almost a thousand kind of plants with edible foliage has been reported in the literature (Wikipedia, 2015). They are referred to as transient herbaceous plants e.g. lettuce and spinach. However, some woody plants with edible foliage are Adansonia, Aralia, Moringa, Morus, and Toona species. They are less in calories and fat but rich in protein, fibre, vitamin C, pro-vitamin Acarotenoids, folate, Manganese and exceptionally higher vitamin K content (Kessler and Glauser, 2014).

Leafy vegetables can be boiled, stir-fried, casseroled, steamed or eaten uncooked. Besides, it can be shawled with other food ingredients into comestible packages or blended as a green smoothie with fruits and water to enables consumption in large quantities.

2.10.1 Classification of vegetables

Green leafy vegetables are an indispensable component of diets with about sixty or more species of vegetables that have green leaf are eaten in Nigeria (Kubmarawa *et al.,* 2009). Their nutrient contents are generally viable and, in some cases, medicinal with potential health benefits (Otitoju *et al.,* 2012). Vegetables can be classified as follows:

A. Classification by used part:

1. Leafy green: These are succulent leaves and shoots usually harvested for consumption. They are Bitter leaves, fluted pumpkin, cabbage, water leaves and lettuce etc.

2. Vegetables with fruits: These are tender unripe fruits or matured ripe fruits of plants cultivated as green. They include tomato, pumpkin, garden egg, watermelon, okro and so on.

3. Vegetables with seeds: They are seed manufactured vegetables. Examples are melon and watermelon etc.

4. Vegetables with roots: These vegetables bear roots. For instance sweet potato, and carrot etc.

5. Spicy vegetables: These vegetable shoots, leaves, and/or fruits produce flavour or used as a spice. They include onion, garlic and chilli pepper etc.

B. Classification by Seasonality:

1. Rainy season vegetables: These vegetables harvested majorly during the rainy season. They include cabbage, onion, spinach, garlic, lettuce, and carrot etc.

2. Dry season vegetables: These are collected during the dry season. They include tomato, pepper, okra, garden egg, and melon etc.

C. Classification by taxonomy:

These are grouped according to biological family, plant genera and species.

Table 2.3: Classification of vegetables based on taxonomy

Family	Biological name	Common name
Amaranthaceae	*Amaranthus dubius*	Amaranth
	Celosia argentea	Celosia
Cucurbitaceae	*Cucurbita maxima*	Pumpkin
	Cucumis sativus	Cucumber
Malvaceae	*Abelmoschus esculentus (L.)*	Okra
Solanaceae	*Solanum tuberosum*	Irish potato
	Solanum melongena L.	Eggplant
Tiliaceae	*Corchorus olitorius L.*	Jews mallow
Compositae	*Vernonia colorata*	Bitter leaf
	Lactuca sativa	Lettuce
Cruciferae	*Brassica oleraceae*	Cabbage
	Raphanus sativus	Radish
Portulaceae	*Talinum triangulare*	Water leaf
Basellaceae	*Basel larubra*	Indian spinach

D. Classification by frequency of consumption:

1. Regularly consumed vegetables: They are Onion, Celosia, melon, Okra; eggplant, Amaranthus and Pepper etc.

2. Occasionally consumed or Wild vegetables: mushroom (Agaricusspp., *Celosia triguna*Ajefawo) *Basellarubra*(White) – Indian spinach *Basell alba*(Red), *Crassocephall umbiafrae*- Bologi, *C. crepidoidis*–Ebolo

E. Classification by the time of maturity, harvesting and growth:

1. Short growth time: Example includes Amaranthus spp., *Celosia argentea*etc.

2. Harvested over weeks or months: They are Corchorusspp., Solanumspp., tomato, Okro, etc.

3. Vegetables with climbing growth habit: These vegetables possess climbing shoots sticking to walls and poles. For example, fluted pumpkin, snake tomato, Basella spp. etc.

4.Creeping stems vegetables: They are melon, watermelon and cucumber etc.

2.10.2 Vegetable type

A. Local vegetables

These are vegetables associated with warm and humid tropics and well adaptable to hot climates and weighty rainfalls. They are often eaten as supplements to the starchy staple foods and also an inexpensive source of protein and micronutrients. They are cultivated in traditional settings and protuberant in compound/backyard farms. Besides, women usually play a crucial role in their production, processing and marketing with low capital requirement and high profitability.

B. Foreign vegetables

They thrive in the temperate region and/or germinate in a high altitude climate that could be found in the highlands of Adamawa and Jos state in Nigeria. Foreign vegetables are quite famous inurban areas. They include; lettuce, cabbage, and Irish potato etc. Their cultivation requires special methods entailing tediousness. They are unique in texture, taste and requires special culinary skills preferable in most homes in the tropics. The inability to cultivate them via mixed farming, with poor seed formation and short-lived storage capacity in warm conditions makes the demand for exotic vegetable quite poor and infrequent.

2.10.3 Importance of vegetables in the human diet

Vegetables provide almost all nutrients deficient in other food components, largely desirable to have balanced diets and avoid shortages of nutrients. They supply minerals; particularly Fe and Ca. They are also good sources of vitamins A, B, and C (e.g. sweetcorn, celosia, waterleaf, bitter leaf etc.) useful in reducing predisposition to infection. In some cases, vegetables have high reserves of carbohydrate as carrot, potatoes, sweet corn among others. The peas and green beans can also serve as a basis of rich protein in human diets and improves the palatability of staple foods. Vegetable aid digestion.

CHAPTER THREE

MATERIAL AND METHODOLOGY

3.1 Survey study design

The experimental design adopted for the consumption study was descriptive cross-sectional while the analytical component was experimental in design

3.2 *Cirina forda* survey location

The Cirina forda consumption survey for this study was carried in two Local Government Areas (LGAs)namely Saki East and Iseyin and was purposively selected for the study. Chemical analysis of the samples was carried out at the Human Nutrition and Dietetics Department of the University of Ibadan, andInstitute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan.Rats studies were carried out attheanimal houses of both Departments of Animal Science, and Veterinary Physiology and Biochemistry, University of Ibadan. Samples preparation for acceptability test was carried out in the kitchen of Human Nutrition and Dietetics Department, while microbial evaluation of the dried *Cirina forda*was done at Microbiology Department, University of Ibadan.

3.3 Study participants

The study population for the field consumption survey of *Cirina forda* involved healthy volunteers living within Saki East and Iseyin Local Government Areas who gave their consents to participate in the study.

3.4 Sample Size Determination

Kasiulevicius *et al.,*(2006) formula was used in calculating the sample size:

$$n = \frac{Z^2 (p)(1-p)}{e^2} = \frac{(1.96)^2 x (0.5) x (1-0.5)}{(0.05)^2} = \frac{0.9604}{0.0025}$$

n= 384

Where, Z = Z score value at 95% Confidence Interval (C.I) = 1.96,

p = Prevalence at 50% of *Cirina forda* consumption,

e = Precision at 0.05,

n= Sample size.

Thus, the calculated sample size was 384. The sample size was adjusted to cater for 20% attrition and a total of 502 respondents were recruited for the survey at the two local Government Areas.

3.5 Sampling technique

A 4-stage sampling procedure was adopted for this study which involved,

First stage: The selection of the two Local Government Areas. The selection was purposively done based on areas where *Cirina forda* larva consumption is popular, available and widely consumed among the populace in Oyo State.

Second stage: This involved stratifying each Local Government Area (LGA) i.e. Saki East and Iseyin into 3 rural and 2 urban wards.

Third stage: This stage involved choosing a community from each of the selected wards listed. This implies that five communities were selected from each Local Government areas involving 2 urban and 3 rural wards.

Fourth stage: There was the selection of eligible participants in the communitiesthrough snowballing based on if the respondents know and/or have consumed *Cirina forda* larva before.

3.6 Inclusion criteria

All consenting participants were healthy volunteers or respondentsliving in communities within Saki East and Iseyin LGAs, who had consumed *Cirina forda* larva before.

3.7 Exclusion criteria

Non-consenting participants and those who lived in these communities and did not know anything or much about *Cirina forda* larva consumption.

3.8 Data collection instruments

Data was collected by using a pretested, semi-structured and interviewer-administered questionnaire on socio-demographic characteristics, consumption pattern and frequency of *Cirina forda*,awareness of the nutrient benefit, availability, acceptability, limitation and restriction on *Cirina forda* consumption.

3.9 Sample collection

Samples of *Cirina forda* larva that was used for chemical, toxicological and microbiological analysis were purchased from markets in Saki (Nigeria sample), Bodija (Ghana sample) and Bobo Diollaso (Burkina Faso sample) and analysed for their nutrient composition while the remaining samples were wrapped in air-restricted cellophane bags and stored at -4°C for further use during the study.

The vegetable leaves and other ingredients used for soup preparation were obtained at Bodija market Ibadan, and the soups were preparedat the Dietetics kitchen, Department of Human Nutrition, University of Ibadan.

3.9.1 Soup standardization

All the ingredients used for the soups were standardized by weighing the items on a weighing balance (ScoutPro, Ohaus) and water was measured using a graduated cup. The weight of the empty pot used was determined using a weighing scale (CAMRY Emperor, China) and the yield was by weighing the pot and its content on the weighing scale.

The recipes collected were standardized using the modified National Food Service Management Institution (NFSMI, 2010) method, which includes three phases: recipe verification, product evaluation and quantity adjustment. In this study, only the two major stages of recipe verification were carried out. This was done for one recipe at a time to find out the recipe contained the following information

a. Recipe title
b. Recipe category
c. Ingredient
d. Weight/Volume for each ingredient
e. Preparation instructions
f. Cooking temperature and time
g. Service size
h. Recipe yield
i. Equipment and utensil used

3.9.2 Methods of Soups Preparation

Water was used to wash the *Amaranthus hybridus*leaves to remove sand and pebbles and then sliced. Three hundred and fifty grammes (350g) of*Cirina forda* larvae were measured, rinsed and softened by soaking in hot water. The softened sample was divided into two portions of 175g each for the preparation of *Efo riro* and Egusi soups. The cooking equipment usedwas stainless steelpot, spoon, and cooking gas.

Efo riro *Cirina forda* larvae enriched soup:

A pre-weighed stainless steel pot was put on cooking gas. Then, 50g of palm oil was added and allowed to fry for 2 minutes. Exactly 14g Pepper and 4g, onions were added and allowed to fry for five minutes. Approximately 4.5g of salt and two bouillon cubes (5g) were added to taste and then 175g of *Cirina forda* larvae were added. Exactly 350ml of water was added to the content in the pot and allowed to seethe for twenty minutes, followed by the addition of 25g of sliced *Amaranthus hybridus* (*Efo tete*) leaves, and allowed to steam for 5minutes. The cooking pot was then removed from the cooking gas, allowed to cool. The pot and its contents weighed to determine the yield. The average yield was 850.21g (Table 3.1)

***EgusiCirina forda* larvae enriched soup:**

A pre-weighed stainless steel pot was put on cooking gas. Then, 50g of palm oil was added and allowed to fry for 2 minutes. Grounded egunsi powder was made into a slurry with 25ml of water and poured into the fried palm oil in the pot. Exactly 14g Pepper and 4g of onions were added and allowed to fry for five minutes. Approximately 4.5g of salt and two bouillon cubes (5g) were added to taste and then 175g of *Cirina forda* larvae were added. Exactly 400ml of water was added to the content in the pot and allowed to seethe for twenty minutes, followed by the addition of 25g of sliced *Amaranthus hybridus* (*Efo tete*) leaves, and allowed to steam for 5minutes. The cooking pot was then removed from the cooking gas, allowed to cool. The pot and its contents weighed to determine the yield. The average yield was 925.21g (Table 3.2)

***Efo riro* soup:**

A pre-weighed stainless steel pot was put on cooking gas. Then, 50g of palm oil was added and allowed to fry for 2 minutes. Exactly 14g Pepper and 4g, onions were added and allowed to fry for five minutes. Approximately 4.5g of salt and two bouillon cubes (5g) were added to taste. Exactly 250ml of water was added to the content in the pot and allowed to seethe for twenty minutes, followed by the addition of 25g of sliced *Amaranthus hybridus* (*Efo tete*) leaves, and allowed to steam for 5minutes. The cooking pot was then removed from the cooking gas, allowed to cool. The pot and its contents weighed to determine the yield. The average yield was 625.21g (Table 3.3).

Melon (Egusi) soup:

A pre-weighed stainless steel pot was put on cooking gas. Then, 50g of palm oil was added and allowed to fry for 2 minutes. Grounded egunsi powder was made into a slurry with 25ml of water and poured into the fried palm oil in the pot. Exactly 14g Pepper and 4g of onions were added and allowed to fry for five minutes. Approximately 4.5g of salt and two bouillon cubes (5g) were added to taste. Exactly 250ml of water was added to the content in the pot and allowed to seethe for twenty minutes, followed by the addition of 25g of sliced *Amaranthus hybridus* (*Efo tete*) leaves, and allowed to steam for 5minutes. The cooking pot was then removed from the cooking gas, allowed to cool. The pot and its contents weighed to determine the yield. The average yield was 645g (Table 3.4).

3.10 Ethical Consideration

The ethical consideration certificate was obtained from the Joint Institutional Review Board of the University of Ibadan/ University College Hospital, Ibadan (UI/EC/16/0276) while the Animal Care and Use Research Ethics Committee of the University of Ibadan approved the animal model experimentations (UI-ACUREC/19/0034). Informed consent of individual respondent recruited for questionnaires administration and the untrained panellists drawn from the University of Ibadan was sought before the commencing of each section of the study.

Table 3.1: Ingredient Used for vegetable soup enriched with *Cirina forda* larva

Ingredients	Weight/Volume	Local Measurement	Variationin Quantity	Optional Ingredient
Pepper(Fresh)	10g	4 pcs	8-16g	Dry pepper
Palm oil	50g		45-75g	G/nut oil
Onion	4g	I small bulb	3-7g	
Salt	3-5g	½ teaspoon	2-6g	
Vegetable (Efo tete)	20-25g		22-30g	
Maggi cube	5g	2 cubes	2-6g	
Water	350ml	2 small cup	300-500ml	Royco
Dried *Cirina forda*	175g	2empty-milk tin	150-200g	

Table 3.2: Ingredients Used for Egunsi soup enriched with *Cirina forda* larva

Ingredients	Weight/Volume	Local Measurement	Variation in Quantity	Optional Ingredient
Egusi	50g	1 empty milk tin		
Pepper(Fresh)	10g	4 pcs	8-16g	Dry pepper
Palm oil	50g		45-75g	G/nut oil
Onion	4g	I small bulb	3-7g	
Salt	3-5g	½ teaspoon	2-6g	
Vegetable (Efo tete)	20-25g		22-30g	
Maggi cube	5g	2 cubes	2-6g	
Water	400ml	2 small cup	380-420ml	Royco
Dried *Cirina forda*	175g	2empty-milk tin	150-200g	

Table 3.3: Ingredients Used for Vegetable soup

Ingredients	Weight/Volume	Local Measurement	Variation in Quantity	Optional Ingredient
Pepper(Fresh)	10g	4 pcs	8-16g	Dry pepper
Palm oil	50g		45-75g	G/nut oil
Onion	4g	I small bulb	3-7g	
Salt	3-5g	½ teaspoon	2-6g	
Vegetable (Efo tete)	20-25g		22-30g	
Maggi cube	5g	2 cubes	2-6g	
Water	250ml	2 small cup	220-270ml	Royco

Table 3.4: Ingredients Used for Egunsi soup

Ingredients	Weight/Volume	Local Measurement	Variation in Quantity	Optional Ingredient
Egusi	50g	1 empty milk tin		
Pepper(Fresh)	10g	4 pcs	8-16g	Dry pepper
Palm oil	50g		45-75g	G/nut oil
Onion	4g	I small bulb	3-7g	
Salt	3-5g	½ teaspoon	2-6g	
Vegetable (Efo tete)	20-25g		22-30g	
Maggi cube	5g	2 cubes	2-6g	
Water	250ml	2 small cup	380-420ml	Royco

3.11 Chemical analysis

3.11.1 Proximate composition

3.11.1.1Dry matter and moisture determination (AOAC Official Method 967.08)

Two grammes (2g) of the sample was weighed into a pre-weighed crucible (W_0) and both sample and crucible (W_1) moved into the oven set at 105^0C till stable weight was got, cooled to room temperature and weighed (W_2).

Weight of empty crucible = W_0

Weight of crucible and sample = W_1

Weight of crucible and oven-dried sample = W_2

% Dry Matter (%DM) = $[(W_2 - W_0) \div (W_1 - W_0)] \times 100$

% Moisture = $[(W_1 - W_2) \div (W_1 - W_0)] \times 100$

or% Moisture = 100 – % DM.

3.11.1.2 Crude protein determination (AOAC Official Method 988.05)

Using the Kjeldahl procedure/technique, crude protein contents of the sample was evaluated through Digestion, Distillation and Titration.

The $\%N_2$ in the titration analysis was calculated using the formula;

$\%N_2$ = (Titre value) x (Normality/Molarity of HCl) x (Atomic mass of N_2)

x (Volume of the flask containing the digest) x 100

Crude protein (CP) composition was derived by multiplying $\%N_2$ with a constant factor of 6.25 i.e. **%CP** = $\%N_2$ x 6.25.

3.11.1.3 Fat or Ether extract determination (AOAC Official Method 2003.06)

Weighed 1g dried sample into thimble from fat-free extraction (lightly pugged with cotton wool) and placed in the extractor with a reflux condenser and previously oven-dried, cooled and weighed 250ml soxhlet flask (W_0) with three-quarter of its volume filled with petroleum ether (boiling point: 40^0-60^0C) and gently boiled on a heater for 6hrs (withthe condensation of ether vapour with continuous running water from the tap). Distillation resumes (after replacing the extractor, flask and condenser) till the flask is essentially dry (containing fat or oil), detached, the exterior cleaned and oven-dried to constant weight (W_1). % fat and oil are estimated as:

% Fat and Oil $= [(W_1 - W_0) \div (\text{Sample weighttaken})] \times 100$

3.11.1.4Ash Determination (AOAC Official Method 942.05)

Weighed 2g sample in a crucible was moved into the muffle furnace at 550ºC temperature for 4hrs to turn to white ash and weighed again (after cooling to about 100ºC in air and at room temperature in a desiccator).

% Ash was estimated as: [(final weight of ash pot - cooling) ÷ (initial weight of sample)] x 100

3.11.1.5 Crude fibre determination (AOAC 958.06)

Weighed 2g sample was added to fibre flask containing 100mL H_2SO_4 – 0.255M and heated under reflux for 1hr with a heating mantle (AOAC, 2006). The residue was oven-dried at 105ºC overnight, cooled in a desiccator, weighed (W_1), and ashed in the muffle furnace at 550^0C for 4hrs, cooled in a desiccator and weighed (W_2). The % fibre was derived as:

% Fibre content $= [(W_1 - W_2) \div (\text{weight of sample})] \times 100$

3.11.1.6Total dietary fibre determination

Weighed 1g of sample into a 50mL centrifuge tube containing 2mL of dimethyl sulphoxide, capped, stirred for 2mins on a magnetic stirrer (to homogenise the sample) and placed in boiling water of beaker with stirrer for 1hr and 8mL of sodium acetate buffer at pH 5.2 was added, pre-equilibrated at 50^0C, vortex mixed (without cooling). Using 50mL 85% ethanol, the residue was washed twice and mixed by inversion on a magnetic stirrer to hang up the residue, the supernatant decanted adding 40mL acetone to wash the residue, stir for 5mins and centrifuged at 1500g for 10mins, decanting the supernatant and tube was suspended in a beaker of water at 65^0C-75^0C with continuous stirring until the residue was dry.

% total dietary fibre (TDF) was estimated as thus:

% total dietary fibre = [(Weight of residue) ÷ (Weight of sample)] x 100

3.11.1.7Soluble dietary fibre determination

The residue obtained from Total Dietary Fibre washed with 10mL distilled water preheated to 70^0C. The filtrate and washings were transferred to a 500mL beaker. 4 x 280mL of 95% ethanolic solution was added and preheated to 60^0C to precipitate the soluble dietary fibre (SDF) for 1hr and filtered through a suction crucible fitted to a suction pump. The residue in the crucible was eroded successively with 30ml each of 78%ethanol, 95% ethanol and acetone respectively to remove any trace of lipids.

The crucible + residue was dried overnight @ 103^0C in an air-dry Gallenkamp oven.

%SDF = [(weight of crucible and residue – weight of empty crucible) ÷ (Weight of sample)] x 100

Also,

% Insoluble Dietary Fibre (IDF) = %TDF - %SDF.

3.11.1.8 Carbohydrate by the difference

Carbohydrate determination by difference was completed by removing the sum of %moisture %crude protein, %ether extract, %crude fibre and %ash from a hundred percent.

E.g. 100% – (%moisture + %crude protein + %ether extract + %crude fibre + %ash).

3.11.2 Determintion of minerals (AOAC, 975.11)

3.11.2.1 Calcium, Potassium and Sodium estimation

From the digested samples, the ash obtained was poured and boil in 5ml 2M HCl and heated to boil, filtered into 100 ml flask and read in Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element. The formula below was used to calculate the concentration of each of the element:

= [meter reading (MR) **x** slope **x** dilution factor] ÷ 1000

NB: the above formulae (excluding the division by 100) will return the concentration of minerals in parts per million (ppm or mg/kg). However, the % concentration would be returned upon dividing with 10000.

3.11.2.2Phosphorus determination (Spectrophotometric method) (AOAC, 975.16)

Using the Vanado-molybdatecolourimetric or spectrophotometric method we determined phosphorus by treating ash sample with 2M HCl solution following Ca determination method (as indicated above) pipetting10mL of the filtrate into 50mL standard flask containing 10mL of vanadate yellow solution added and made up to mark with distilled water, corked and tolerated for 10mins until the development of yellow colour and optical density (OD) or absorbance of the yellow solution was obtained using Spectronic 20 spectrophotometer or colourimeter at 470nm wavelength to determine phosphorus concentration. % percentage phosphorus was determined as mentioned below:

%Phosphorus=[Absorbance **x** Slope **x** Dilution factor] ÷10000

3.11.2.3Selenium, Magnesium, Copper, Manganese, Iron, Zinc determination (AOAC, 975.23)

Digest of the sample ash from above were washed (using deionized water) into 100ml volumetric flask and made up to mark and aspirated into 200 Atomic Absorption Spectrophotometer through the suction tube of the buck.Each of the trace elements was read using appropriate fuel and oxidant mixtures at unique specific wavelengths and hollow cathode lamps.

Meter readings of individual element was utilized in determining the concentration of each element with the formulation below:

ppm or mg/kg (any element) = Meter reading **x** Slope **x** dilution factor.

% (any element) = **ppm or mg/kg÷** 10000

3.11.3 Determination of vitamins

3.11.3.1 Determination of Vitamin A

Weighed 2g of the sample into a reflux flask (flat bottom) containing 10ml of distilled water, carefully shaken to form a paste and 25ml of alcoholic KOH solution was added attaching a reflux condenser, heated in boiling water for 1hr while shaking.The mixture was easily cooled with 30 ml of water and the collected hydrolysate was transferred to a separator funnel. Using 250ml chloroform, the solution was extracted three times and water traces were removed by adding 2g anhydrous Na_2SO_4, filtered into a volumetric flask of 100 ml and made using chloroform to mark up. On the Spectrophotometer (MetrohmSpectronic 21D Model), sample absorbances and standards were read at a wavelength of 328nm.

CALCULATIONS:

Vitamin A (µg/100g) =[(Absorbance of the sample) **x** (dilution factor)] ÷ (Weight of sample)]

CONVERSIONS:

6µg ß-carotene ≈ 1 retinol

12µg of other biologically active carotenoids ≈ 1 retinol

1 retinol equivalent of Vitamin A activity ≈ 1µg retinol.

1 retinol ≈ 3 IU (International Unit).

3.11.3.2Determination of Vitamin B1 (Thiamine)

A gramme of the sample was measured into a 100ml volumetric flask; 25mL of 0.1MH_2SO_4 was added and carefully swirled. To rinse any adhering sample particle off the flask, an additional 25ml of 0.1M H_2SO_4 was applied. To ensure a complaint about the breakdown of the sample in the acid, the flask was put in a boiling water bath.The flask was shaken frequently in the first 5 minutes and subsequently, every 5 minutes for 3 minutes and 5ml of taka-diastase in 0.5M Sodium acetate solution was added, flask cooled below 50^0C, stoppered, kept at 45^0C – 50^0C for 2hrs and made up to 100mL and filtered (after thorough mixing), filtrate preserved (after discarding the first 10ml). 10ml of the filtrate was pipette into a 50mL volumetric flask containing 5ml of acidic KCl, thoroughly shaken and 10mg/mL– 50mg/mL standard thiamine solution were prepared from 100mg/mL stock.

Sample and standards absorbance reading on fluorescent UV spectrometer (Cecil A20 model) at 285nm wavelength were recorded.

Vitamin B_1in mg/100g was deduced using the formula:

[(Absorbance of sample) **x** (average gradient) **x** (dilution factor)] ÷ (weight of sample)]

3.11.3.3Vitamins B_2 (Riboflavin) determination

A sample of 1g was weighed into a 250ml volumetric flask containing 5ml of Normal HCl and 5ml dichloroethene and shaken adding 90ml of deionized water.The mixture was heated for 30 minutes in a steam bath (to mine riboflavin), cooled, made up of deionized water to label and then filtered (removing the first 20ml of the aliquot). Also, 2 ml of filtrate was pipetted into a 250 ml flask and produced with deionized water up to volume.

By dissolving 0.05mg Riboflavin into 100mls of distilled water, standard solutions were prepared. To achieve the equivalence, different standard solution concentrations of between 0 to 5 ppm were prepared from above. Sample and standards absorbance reading on fluorescent spectrometer at 460nm wavelength were recorded. Vitamin B_2 concentration was calculated as follows:

Vitamin B_2 (mg/100g) =

[(Meter reading of sample) **x** (standard Eq. factor) **x** (dilution factor)] ÷ (Weight of sample)]

3.11.3.4Vitamin B3 (Niacin or Nicotinic Acid) determination

A sample of 5g was blended and 100ml of distilled water added to melt all nicotinic acid or niacin present in the solution. Furthermore, 5ml of the resultant solution was pipetted into 100ml volumetric flask and made up to volume using distilled water and used in preparing 10 – 50ppm of niacin stock solution. Sample and diluted stock absorbances (to obtain gradient factor) reading on a fluorescent spectrophotometer at 385nm wavelength were recorded and

Niacin concentration (mg/100g) in the sample was:

[(Absorbance of sample) **x** (dilution factor) **x** (gradient factor stock solution)] ÷ 10

3.11.3.5Vitamin B6 (Pyridoxine) determination

A sample of 1g was weighed into a 100ml beaker containing 0.5g of NH_4Cl, 45ml chloroform and 5ml absolute alcohol to extract pyridoxine (Vit.B6), thoroughly shaken (for 30mins) and 5ml of distilled water was added to the mixture in a separator funnel to distinguish the aqueous chloroform layer containing the pyridoxine and filtered into 100ml flask and made up to volume with chloroform. 0-10ppm pyridoxine standard was prepared from 100ppm stock standard solution of pyridoxine, treated to obtain the gradient factor and absorbance of the resultant yellowish colour solution was measured on Cecil 505E Spectrophotometer at a 415nm wavelength.

Vit B6 (mg/100g) was calculated as:

[(Absorbance of sample) **x** (dilution factor) **x** (gradient factor)] ÷ [weight of sample **x** 10]

3.11.3.6Vitamin C (Ascorbic Acid) determination

A sample of 10g slurry was weighed, diluted to 100mL using 3% meta-phosphoric acid solution (0.0033M EDTA) and filtered. 10ml filtrate was pipetted and titrated with 2, 6, di-chlorophenol-indophenol standardized solution to a faint pink endpoint and **ascorbic acid concentration was calculated as below;**

$$[(V \times T) \div W] \times 100$$

Where; V = volume (ml) of dye used for titration of an aliquot of the diluted sample.

T = ascorbic acid equivalent of dye solution expressed (mg/ml of dye).

W = weight of sample (g) in aliquot titrated.

3.11.3.7Vitamin E (Tocopherol) determination

Weighed 1g of the sample intoa conical flask of 250ml containing 10mL absolute alcohol and 20mL 1M alcoholic sulphuric acid, refluxed and cooled for 45mins. After cooling, transfer the mixture into a 250mL separating funnel and protect with aluminium foil (after adding 50ml of distilled water). Using 5 x 30mL dimethyl ether with the mixture, unsaponifiable matters were extracted, washed to remove acid, dry-evaporated at lower temperatures and residues were

immediately dissolved in 10ml absolute alcohol. Aliquots arising from the sample and standards (0.3-3.0mg vitamin E) were transported to 20ml volumetric flask with 5ml absolute alcohol, carefully adding 1ml concentrated HNO_3 and flasks were suspended in the water bath at 90^0C for 3mins as soon as the alcohol begins to boil, rapidly made to cool under running water and adjusted to absolute alcohol with a volume. The absorbance of the resultant mixture (against blank of 5ml absolute alcohol and 1ml concentrated HNO_3 treated similarly) was measure at 470nm wavelength and **vitamin E (µg/100g) was evaluated as:**

[(Absorbance of sample) **x** (dilution factor) **x** (gradient factor)] ÷ weight of sample

3.11.4.0Amino Acid Profile

Sample preparation for amino acid analysis

In an extraction thimble, a sample of 2.5g was weighed and fat extracted (for about 5-6hrs) with a chloroform/methanol (2:1, v/v) mixture with Soxhlet apparatus (AOAC, 2005).

Hydrolysis of samples

30mg of defatted samplewas weighed into glass ampoules with 7ml 6M HCl expelling oxygen by ensuring the nitrogen gas pass into the sample. Bunsen flame was used to seal glass ampoules and placed in an oven (105±5°C) for 22hrs but later allowed to cool and the content filtered to eliminate the humid. The filtrate was evaporated to dryness in a rotary evaporator at 40 ° C under vacuum and the resulting residue was dissolved with a 5 ml acetate buffer (pH 2.0) and deposited in a deep freezer in a plastic specimen container.

Amino acid analysis

Using ion-exchange chromatography (IEC) (FAO/WHO, 1991) with Technicon Sequential Multi-sample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York) at a gas flow rate and temperature of 0.50ml/min and 60°C respectively with reproducibility consistent within ±3%, net height of each peak produced by TSM chart recorder (with nor-leucine as internal standard) was measured and calculated and amino acid profiles were reported.

Tryptophan

Weighed samples placed in polypropylene tubes after addition nor-leucine (as internal standard), were hydrolysed in 4.67M KOH containing 1% (w/v) thiodiglycol for 18hrs at 110°C and KOH neutralized using 2.4M perchloric acid, adjusting the supernatant to pH 3.0 with acetic acid. 20 µl aliquot of the hydrolysed sample was subjected to derivatization as above and the amino acid solution was supplemented with tryptophan. Tryptophan determination was quality assured by demonstrating the method generated a similar number of tryptophan residues for egg white lysozyme. Waters C18 reversed-phase column (3.9 x150 mm) (Waters Milford, MA), the solvents and gradient conditions as described by Hariharan *et al.,* (1993) were used to quantitate tryptophan levels and elution protocol was applied to adequately distinct tryptophan from ornithine.

Amino Acids Profile Determination(Thin Layer Chromatography and Modified Colorimetric Techniques)

Extraction of amino acids

Samples of two grammes (2g) were [weighed using a digital chemical balance – model OHAUS precision plus) blended, transferred into 250mL beaker of phosphate buffer solution (0.2M;20ml) pH 7.0, stirred for around 3mins and centrifuged at 200rpm for 10mins. The supernatant was (decanted using a separating funnel) and shaken thrice with a 10ml portion of petroleum ether to confiscate any form of organic pigments and the top phase was discarded retaining the aqueous phase where protein and amino acids are trapped. 5ml 10% w\v trichloroacetic acid (TCA) for 5mL extract was added to the precipitate protein, shaken and stored in the freezer for 10mins thereby forming a precipitate removed by centrifugation and the filtrate was used for the acid profile analysis.

Chromatographic analysis of amino acids (TLC technique)

Amino acids were separated using the thin-layer chromatography method. 50µL extracts were spotted on Avicel microcrystalline cellulose thin layer plates (Whatman analytical plates) with a 20µL reference standard mixture. The reference mixture included; lysine, histidine, phenylalanine, methionine, glycine, cysteine, proline, leucine, isoleucine, threonine, tyrosine, valine, arginine, tryptophan, and glutamic acid at a concentration of 0.1% (w\v). One

dimensional ascending chromatography was explored, using an n-butanol-glacial acetic acidic water solvent system at 4:1:2 (v\v) ratio. After 4hrs of separation, chromatograms were aired-dried and the amino acids were located by spraying locating reagent (0.2% (w\v) of ninhydrin in ethanol) and again air-dried and subsequently oven-dried at 100^0C for 5mins to identify the spots using the spotted reference standard equivalence.

Quantitative determination of amino acids profile

Modified guide strip colourimetric technique for estimating amino acids was used to quantify amino acids profile. Thin layer chromatographic plates were developed for detecting amino acids positions in unsprayed plates and squares containing the amino acids were cut out, eluted with 5mL distilled water at 70^0C for 2hrs; and cellulose powder was detached by centrifugation at 5000 rpm for 5mins decanting and preserving the supernatants for the colourimetric evaluations.

Colourimetric analysis of amino acids profile

Extracts gotten were used to quantitate amino acids profiles through the modified ninhydrin colourimetric analysis technique. To 1mL of diluted extracts of each amino acid was added 0.5mL cyanide–acetate buffer pH (5.4) and 0.5mL 3% (w\v) ninhydrin in methylcellosolve and heated in boiling water bath at 100^0C for 15mins. The mixture was removed from the water bath, 5.0mL Iso-propyl alcohol-water mixture (ratio 1:1) was added as diluents, vigorously mixed and then cooled to room temperature (25^0c). Using UV\Visible spectrophotometer, the optical density of samples and blank (distilled water) was determined at 570nm, and amino acid content of each sample calculated from the standard curve of known concentration of leucine (10mg\mL).

% amino acid = [(Absorbance of sample) **x** (dilution factor) **x** (gradient factor)] ÷ 10000

3.11.5.0Spectrophotometric determination of Fatty Acids 3.11.5.0

Two gramme, 2g sample was weighed into 100mL conical flask with 20mL of Benzene, thoroughly mixed to extract fatty acids, transferred to 250mL separator funnel to distinct benzene from the aqueous extract and 5mL aliquot benzene extract was pipetted into a 15mL test-tube

with 2mL 10% Copper acetate for blue colour development. Standard solutions for individual fatty acids were primed in the range 0-10ppm from 100ppm stock solution of each fatty acids and absorbance of samples and standards read on a Spectrophotometer at a defined wavelength for individual fatty acid as listed below: Lauric Acid(640nm), Stearic Acid (650nm), Palmitic Acid (630nm), Arachidonic(690nm), Oleic Acid (670nm), Linoleic Acid (660nm), Linolenic Acid (680nm), Ricinoleic Acid (610nm), Dihydroxy Stearic Acid (655nm).

%Fatty Acid =

[(Sample absorbance) **x** (DF) **x** (specific fatty acid gradient factor)] ÷ [sample weight **x** 10000]

Where **DF** = Dilution Factor.

3.11.6.0Anti-Nutrients Determination

3.11.6.1Phytate determination

Weighed 2g of sample into 250mL conical flask with 100ml 2% HCl, soaked for approximately 3 hours, filtered through a double-layer-hardened filter paper and measured 50mL of the filtrate into 0.50mL conical flask with 107mL distilled water. Add 10ml of 0.3% Ammonium cyanide solution to the solution and titrate with $FeCl_3$ standard (containing 0.00195g/mL of iron) to discern a persistent (for 5mins) slightly brownish-yellow end-point.

Per cent (%) phytic acid was calculated as:

[Titre value **x** 0.00195 **x** 1.19 **x** 100 **x** 3.55] ÷ [sample weight]

3.11.6.2Saponin determination

One gramme, 1g of finely ground sample was weighed into a 250mL beaker with 100mL isobutyl alcohol, thoroughly shaken on UDY shaker for 5hrs and filtered through a Whatman No1 filter paper into a 100mL beaker containing 20ml of 40% saturated magnesium carbonate solution to obtain saturated $MgCO_3$ and filtered for the second time through a Whatman No1 filter paper for a clear colourless filtrate. In a 50 mL volumetric flask with 2 mL 5% $FeCl_3$

solution, 1 ml colourless solution was pipetted, made up to label with distilled water and allowed to stand for blood-red colour production for 30 minutes.In a Jenway V6300 Spectrophotometer at 380nm wavelength, 0-10ppm standard saponin solutions were prepared from saponin stock solution, similarly treated with 2mL of 5% FeCl3 solution as above and the absorbance was measured after colour growth.

%Saponin is calculated as:

= [(Sample absorbance) **x** (dilution factor) **x** (gradient factor)] ÷ [sample weight **x** 10000]

3.11.6.3Tannin determination

Weighed 0.20g of sample into a 50mL beaker with 20mL 50% methanol, covered with parafilm, suspended in a water bath at 77-80^{o}C for 1hrs, shake thoroughly and filtered into a 100mL volumetric flask with 20mL water and 2.5mL folin-Denis reagent with 10mL 17% Na_2CO_3 were added shaken properly. The mixture was made up of water and allowed to stand for 20mins until bluish-green colour showed up at the end of range 0-10ppm.

The absorbance of samples and standard solutions containing tannic acid wasmeasured on a Spectronic 21D spectrophotometer at 760nm wavelength.

%Tannins was calculated as:

= [(Sample absorbance) **x** (dilution factor) **x** (average gradient factor)] ÷ [Sample weight **x** 10000]

3.11.6.4Determination of Trypsin Inhibitor Activity

Weighed 0.2g of defatted sample into a centrifuge tube containing 10mL of 0.1M phosphate buffer and mixed well for 1hr. The centrifuged the mixture at 5000rpm for some minutes. Then, the filtered the solution into a 250mL conical flask. Pipette 0.2, 0.4, 0.6, 0.8, and 1.0mL of stock trypsin solution were also pipetted in triplicate as followed in the standard TIA determination procedure. Filtrate absorbance of both standard trypsin solution and samples was measured on a

Spectrophotometer at a 280nm wavelength observing the difference between absorbance of stock and sample trypsin filtrates.

TIU for each sample was presented as:

= (Change in sample absorbance extract) ÷ [0.01 **x** protein in sample (mg)]

3.11.6.5Oxalate estimation/determination

Two grammes, 2g sample was boiled in 40mL water for 30mins in a reflux condenser, 10mL 20% Na_2Co_3 was added, boil again for 30mins, filtered and washed with hot water until the absence of alkaline reaction in the wash water. HCl (1:1) was added dropwise and stirred until the final acid concentration after neutralization is about 4% when a heavy precipitate appears (which is allowed to flocculate). The extract was carefully filtered into a 250ml flask and made up to mark, keep overnight, and filter the supernatant liquid through a dry filter paper into a dry beaker. Measure aliquot of the filtrate in a 400mL beaker, dilute with water to 200mL, re-acidifying with lactic acid. 10mL of 10% $CaCl_2$ solution was added and stirred (in a cold medium) to allow the appearance of calcium oxalate precipitate and allowed it to settle overnight. The clear supernatant liquid was carefully decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. The precipitate was dissolved in HCl (1:1) and oxalic acid was re-precipitated adjusting pH with NH_4OH solution and contents were boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution.

Calculation

1ml 0.05N $KMNO_4$ = 0.00225 anhydrous Oxalic Acid

% Oxalic Acid= [(Titre value **x** 0.00225) ÷ 2] **x** 100

= Titre value **x** 0.1125

3.12.0 Toxicological study of *Cirina forda*larva

Randomised experimental design was the procedure used for selecting eighteen (18) female Wistar strain rats between 8 and 12 weeks old within the weight range of 100-150g were procured from the Physiology Department, University of Ibadan. The rats were allowed to acclimatize in the cages for fourteen days and on commercial rat pellets ad libitum (Top feed starter Marsh) and clean tap water before randomization into groups.

3.12.1 Preparation of *Cirina forda* larva liquid extract and *Cirina forda* supplemented feeds based on modifiedOrganization for Economic Cooperation and Development(OECD Guideline 432, Adopted, 2001)

According to the modified (OECD Guideline 432, Adopted, 2001), dried *Cirina forda* larvae were pulverized with a blender to a fine powder and 50g of pulverized powder was weighed into a beaker containing 100 mL distilled water to soak the powder for several hours. The Marsh was decanted thoroughly inside the cheese-cloth sieve for the extraction of the filtrate (Liquid extract) in the marsh. The flow-chart of the procedure was presented as follows:

Figure 3.1: A flow diagram of *Cirina forda* liquid extract(OECD Guideline 432, Adopted, 2001).

Dosage:

The solution of the dosage of 5000mg/kg of the *Cirina forda* solution prepared was kept inside a refrigerator at a temperature < 4^0 C before use.

Feed Preparation:

Formulated diets of 20% and 80% levels of inclusion of *Cirina forda* powder were prepared by blending 20%*Cirina forda*powder with 80% commercial feed and 80% *Cirina forda* powder with 20% commercial feed respectively. Meanwhile, the control is 100% commercial feed pellet.

3.12.2 Administration Procedure

A. Oral Acute Toxicity Test: Single Dose Administration (OECD Guideline 432, Adopted, 2001)

Two groups with 3 rats each: group 1 (*Cirina forda* liquid extract group - experimental) and group 2 (Control group) were adopted for this study. The rats were allowed to fast overnight before, and 2-3 hours after the oral extract administration.*Cirina forda*larva liquid extract dosage of 5000mg/kg of the rat body weight was skillfully injected in a single dose of 1mL/100g by gavage using appropriate intubation cannula for the rats in the experimental group.

Experimental rats were assessed exclusively after dose up at 0, 15, 30mins, 1, 2, 3, 4hrs, and daily for the fourteenth day to discern sign(s) toxicity using modified Hippocratic screen table (Malone *et al.,*1962) and mortality. 15g of commercial rat pellets was administered to each rat in the groups daily, and feeding water was replaced every other day for 14 days. Left-over diets were collected and weighed daily but animal weights were taken once in 3 days.

B. Oral Acute Toxicity Test: Repeated Feed Administration for 14 Days

Three rat groups were used thus: two diets were formulated by adding *Cirina forda* powder at 20% and 80% levels of inclusion to commercial rat pellet and fed to 4 female rats/group as the experimental groups. Another 4 female rats were fed 100% rat pellet (control group). Fifteen

grammes of experimental diets and commercial rat pellet was supplied to each rat/day for 14 days. Feeding water was exchanged every other day for 14 days, left-over diets were gathered and weighed daily but animal weights were taken once in 3 days. The experimental rats were observed individually after dosing at 0, 15, 30mins, 1, 2, 3, 4 hrs daily for 14days to discern clinical traces of toxicityand mortality using the Modified Hippocratic Screen table(Malone *et al.,*1962). Check the Appendix.

3.12.3 Urine collection

The experimental rats' urine samples were collected on the last (14^{th}) day of the experimental feeding before the rats were sacrificed for the urinalysis test. The urine sample from each of the rats was collected by free catch into a sterile collection tube for analysis. The urine dipstick was dipped into each urine sample to generate a semi-quantitative measurement of urobilinogen, protein, pH, blood, ketones, bilirubin and glucose

3.12.4 Blood and Organs Collection for Analysis

The experimental rats were sacrificed on the 14^{th}day via cervical dislocation, and blood samples collected by ocular puncture before surgical dissection for organ harvest. One portion of the blood was collected intobottles containing potassium and ethylenediamine tetraacetic acid(EDTA) for analyses of blood parameters such as packed cell volume, haemoglobin, red blood cell, platelets, white blood cells and mean corpuscular volume, using an automatic haematological machine – Cell-DynTM Abbot, US. Another blood portion was collected in plain bottles, allowed to clot and centrifuged at 3500rpm for 10mins, sera were separated and stored at -4°C before evaluation of biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, total and conjugated bilirubin, serum urea nitrogen and creatinine using commercial kits obtained from Randox Laboratories, UK. The Brain, liver, and kidneys were harvested, weighed, and observed microscopically. Estimation of relative organ and/or bodyweight ratio (ROW) was done as follows:

Relative Organ Weight, ROW=absolute organ weight (g) /body weight (g) on sacrifice day

The Histopathological assessments of harvested organs were conducted at the Veterinary Physiology Laboratory, University of Ibadan. Check section 3.13.5

3.12.4.1 Determination of Haematological Parameters

3.12.4.1.1 Haemoglobin concentration

This was determined by the cyanmethaemoglobin method of Jain (1986).

Reagent

Drabkin reagent was prepared by dissolving 1g sodium bicarbonate, 2mg potassium cyanide and 198mg potassium ferricyanide in distilled water and made up to 1000mL.

Procedure

After haemolysis of the cells, 0.02ml of blood was added to 5mls of Drabkin's solution and mixed well. Solution absorbance was measured using a spectrophotometer at 540nm wavelength after solution stood for 10mins to ensure full-colour development. Drabkin solution was used as blank and the standard was a cyan-methaemoglobin solution.

Calculation

Haemoglobin (Hb) (g/dL)

= [(Absorbance of Test solution) **x** Hb conc. **x** dilution factor] ÷ absorbance of the standard

Where, Hb concentration of standard = 0.0572g/dL

3.12.4.1.2 Packed cell volume (PCV)

The micro-haematocrit technique as described by Jain (1986) was used. The capillary tube was packed with well mixed whole blood, wrapped a one end using plasticine, positioned in the

micro-haematocrit centrifuge and spun at 10000rpm for 10mins. Spun tubes suspended in specially designed haematocrit scale to read **% PCV.**

3.12.4.1.3 Erythrocyte Count

Reagent

Hayem's solution was made up of 0.5g of mercuric chloride, 1.0g sodium chloride and 5.0g of sodium sulphate dissolved in 200mls distilled water.

Procedure

Total erythrocyte count was carried out using the haemocytometer technique described by Jain (1986). 0.5mls of blood containing anti-coagulant (Na EDTA) was drawn into a pipette and diluted to 100 mark. After shaking gently, the solution was put into the hemocytometer and allowed to settle. Counting was done using the objective lens of the microscope (x40). The erythrocyte in 5 of the 25 smallest squares in the central area was counted.

Calculation

Total Erythrocyte per cu mm (u/L) = (Sum of the cells in the five small squares) **x** 10,000

3.12.4.1.4 Leucocyte Count

Reagent

Leucocyte diluting fluid made up of glacial acetic acid, 2mL gentian violet (1% aqueous 1mL; distilled water 100mL).

Procedure

The blood (2 or 3 drops) was diluted with the WBC diluting fluid and after allowing a minute for the leucocyte to settle; the cells were counted under the objective lens of the microscope (x16) from the large corner spaces.

Calculation

Total Leucocyte count per cu mm = Sum of the cells counted x 50

3.12.4.2 Determination of serum biochemical parameters

3.12.4.2.1 Total Serum Protein

This is grounded on the biuret reaction between copper and serum protein in an alkaline medium to form copper-protein complexes. The reaction of copper with the peptide bond of serum proteins leads to the formation of purple colour.

Reagent

Biuret reagent comprised copper sulphate 0.15 (w/v) and sodium hydroxide 1:1 (w/v) with tartrate and iodine added. Albumin (5g/dL) and globulin (3g/dL).

Procedure

The method of Gornall (1949) as described by the Sigma Diagnostic (1990) was used. 5mL of Biuret reagent was added to three different test tubes containing separate solutions of 0.1mL of water (reagent blank), protein (standard solution) and serum sample, stood for 15 minutes at room temperature and absorbance was read on a spectrophotometer at 540nm wavelength.

Serum total protein (g/dL) = [(A test ÷ A standard] **x** 8

Where A test and A standard represented the absorbance of test and standard solutions respectively. The total protein in the standard solution was 8g/dL.

3.12.4.2.2 Serum Albumin

Reagents

1. **Molar Glycine**
   75.07g of glycine was liquefied in 100ml of deionized water

2. **In HCl**

   86mL of conc. HCl was liquefied in 100ml of deionized water

3. **Bromocresol green indicator**

   1.396g of Bromocresol green was dissolved in 100mL of AR grade of absolute alcohol and stowed in dark glassware at ambient temperature.

4. **Standard albumin solution**

   5g of bovine albumin was dissolved in 100mL of deionized water

5. **Working colour reagent (WCR)**

   800mL of deionized water, 94.5mls of molar glycine and 5.5mL of IN HCl and 3.0mls of Bromocresol green solution were made up to 100mL and the PH adjusted to 3.8.

Procedure

The procedure followed was the method of Rodkey (1965) as modified by McPherson *et al.,* (1972). 5.0mL of working colour reagent (WCR) was pipetted in duplicate and to these were added 0.02ml of plasma. The colour change occurred and absorbance was read in a UNICAN spectrophotometer at 635nm. The working colour reagent was the blank and standard albumin solution was used along with the sample.

Calculation

Serum albumin = [(A test ÷ A standard] **x** 5

Where A test and A standard represented the absorbance of test and standard solutions respectively. Albumin in standard solution was and 5g/dL.

3.12.4.2.3 Serum Bilirubin

The bilirubin was coupled with diazotized sulfanilic acid (p-azobenzene sulfonic acid) to form azobilirubin

Reagents

1. The caffeine reagent contained sodium benzoate 38g/l in sodium acetate solution.
2. Alkaline tartrate solution contained sodium potassium tartrate 350g/L in sodium hydroxide solution.
3. Diazo reagent contained a mixture of sulfanilic acid 75mole and sodium nitrite 6.6mole.
4. HCL- 0.05N or 0.05mol/L

Procedure

The procedure was based on the method of Jendrassik and Grof (1938) as modified by Sigma Diagnostic (1990). The test tube for the blank contained 0.5mL Hcl, 1.0 caffeine reagent, 0.5mL diazo reagent and 1.5mL of alkaline tartrate solution. The test tube for total bilirubin contained 0.2ml serum, 1.0 caffeine reagent, 0.5mL diazo reagent and 1.5mL of alkaline tartrate solution. The absorbance of the total bilirubin was read at 600nm with the blank as the reference.

Calculation

Serum total bilirubin (mg/dL) = A **x** 13.2

Where A is the absorbance at 600nm wavelength

3.12.4.2.4 Determination of Activity of Aspartate Aminotransferase (AST)

Reagents

1. **Prepared substrate**
   This was prepared by dissolving 0.2mole of DL- Aspartate and 1.8mmol of α-ketoglutaric acid in a litre of phosphate buffer PH 7.5
2. **Colour reagent**
   - **i.** DNP Solution was prepared by dissolving 20mg of 2,4-dinitrophenyl hydrazine in dilute acid solution
   - **ii.** 0.40N Sodium hydroxide solution. This was prepared by melting 16g anhydrous sodium hydroxide in 100mL deionized water.

Procedure

The procedure used was that described by Reitman and Franke (1957). A test tube containing 1.0mL of the alanine-α-KG substrate was dropped in a water bath at 37°C. Later, a 0.2mL serum sample was added. About 30mins later, 1.0mL of the colour reagent was added, shaken and left at ambient temperature. At 20mins later, 10mls of 0.40N sodium hydroxide solution was added.

The solutions were mixed by several inversions and the absorbances were read at 340nm after five minutes. The activity of the alanine amino-transferase was read from a calibration curve.

3.12.4.2.5 Determination of the Activity of Alkaline Phosphate (ALP)

Reagents

ALP reagent A

This contained diethanolamine 1.214mol/litre in buffer pH 9.8 and 0.607nmol/litre of magnesium ions.

ALP reagent B

This contained 60.8nmol of p-nitrophenyl phosphate in a litre of deionized water.

Procedure

This was as described by Sigma Diagnostics (1990). The ALP reagents A and B were maintained at 30°C in a water bath after their constitution. To 2.7mL of the ALP reagent A was added 0.05mL of the serum sample and mixed immediately by inversion. This was incubated for 1min at 30°C. 0.25mL of the ALP reagent B was then added, mixed immediately and taking the initial absorbance at 405nm with water as a reference. The incubation was continued at 30°C and the absorbance taken again exactly after 1, 2 and 3mins succeeding the initial absorbance readings.

Calculation

ALP activity (U/L) = [A **x** T_v**x** 1000] ÷ [18.45 x LP x S_v]

ALP activity (U/L) =$\frac{[A \times T_v \times 1000]}{[18.45 \times LP \times S_v]}$

Where A = change in absorbance per minute at 450nm

T_v = Total volume (mL)

S_v = Sample volume (mL)

18.45 = mM absorptivity of p-nitrophenol at 405nm

LP = Light path (1-cm)

1000 = Conversion factor of units /mL to /L.

3.13.0 Nutrient Bioavailability Study

Twenty-four 21-day old weanling Wistar rats were purchased from the Physiology Department, University of Ibadan. Each rat was kept separately in a metabolic cage and fed ad libitum with rat pellet for seven days to acclimatise, and then distributed into four groups based on their weights, with a groupweight difference of ±2.2g. The first group was fed with basal diet (Basal group), the second group was fed with *Cirina forda* obtained from Saki (experimental S group); the third group was fed with *Cirina forda* obtained from Ghana (experimental G group); while the fourth group was fed with standard protein diet (Control group). The table for the diet formulation is represented below:

Table 3.5: Rats group diet composition (g/1260g diet)

Feed Component	Basal	Experimental (Saki)	Experimental (Ghana)	Control
Starch	1033.20	790.10	797.30	901.70
Cellulose	63.00	63.00	63.00	63.00
Vegetable Oil	100.80	100.80	100.80	100.80
Vitamin mix	12.60	12.60	12.600	12.60
Mineral mix	50.400	50.40	50.40	50.40
Cirina forda powder	-	242.90	235.80	-
Protein (Casein)	-	-	-	132.3
Total	1260.00	1260.00	1260.00	1260.00
Gross Energy (Kcal/g)	2.60	3.60	3.60	2.90
Nitrogen (%)	0.20	3.00	3.10	2.600

Source: Adepoju and Ajayi, 2016

The feed for all the groups was iso-caloric while the experimental and control diets were iso-nitrogenous. Rats were fed ad libitum with water and 10g of diet every day for 28 days. On daily basis, the leftover feed was collected, weighed and recorded, while rats were weighed weekly.The rats were sacrificed on the 28th day using anaesthesia, and blood samples collected for nutrient bioavailability analysis. Serum retinol was assessed using AOAC(1990) method, while serum ferritin and zinc were analysedby AOAC (2006). The information collected during the feeding experiment was used in the determination of Consumption Index (CI), Growth Rate (GR), Efficiency of Conversion of Ingested food (ECI).

3.13.1 Measurement of Nutritional Indices

The nutritional indices in the study were determined and calculated according to equations described as follow:

Conversion Index, Growth Rate and Efficiency of Conversion of Ingested food calculation by Waldbauer (1968)

Conversion Index,**CI** = C÷ TA;

Growth Rate**, GR** = G÷ TA;

The efficiency of Conversion of Ingested food, **ECI** = [G÷ C] **x** 100

Where:

C = fresh weight of feed consumed.

T = duration of the feeding period.

A = mean fresh weight of the rat during the feeding period.

G = fresh weight gain of the rat

3.13.2. Determination of Protein Efficiency Ratio

Protein efficiency ratio (PER) assess protein nutritional value in an in-vivo assay by gauging rat growth as weight gain/gram of protein fed. The weight of each rat was measured at the beginning of the assay and then rat weight was taken every 7-day interval (weekly) and at the 28th day. The food intake of each animal was recorded for 28 days and PER was obtained as a ratio of average total weight gain and average total protein intake per diet group onthe 28th day.

PER = [Total Weight Gain in Test Group (g)] ÷ [Total Protein Consumed]

The PER value was normalized for the test protein (i.e. comparing the quality of test protein to casein) by assigning casein a **PER of 2.5**.

Adjusted PER = [PER of test protein] ÷ [PER of casein as a control]

3.13.3 Mean Weight Gain

Mean weight gain was evaluated as a difference of the mean final and initial weight of rats in each diet group.

3.13.4 Serum Analysis

After the feeding trial which lasted for 28 days, the blood sample of each animal was taken.

Blood collection from the orbital sinus (ocular bleeding method)

Rats were anaesthetized using diethyl ether. The skin was pulled above and below the eye, to ensure the protruding of the eyeball out of the socket avoiding occlude of the trachea with the thumb. The tip of a fine-walled Pasteur pipette (1-2mm) was inserted into the corner of the eye socket underneath the eyeball, a 45-degree angle toward the middle of the eye socket and rotated between fingers with gentle downward pressure until the vein was broken and blood was visualized entering the pipette and slightly withdrawn to allow blood flow freely into EDTA tubes.

3.13.4.1 Determination of Serum Zinc, Calcium and Ferritin

One millilitre (1mL) of the blood sample was pipetted using a 3mL micropipette into a 30mL digestion tube, 5mL of concentrated HNO_3 (Optima grade), 2mL of concentrated H_2O_2(Hydrogen peroxide), and 13mL of deionized water were added to the digestion tube. The peroxides permitted higher digestion temperatures by reducing the nitric acid vapours as well as removing complex matrix and blood biohazards. The digestion tube was suspended in the appropriate hole of the Digestion Block Heater (TECATOR BD20) and allowed to digest to a clear transparent solution. The transparent solution after cooling down was cautiously transferred to a 50mL volumetric flask and made up to the mark with deionized water. This diluent was used to read for metals like Fe, Ca, Zn and others on a BUCK 211 VGP Atomic Absorption Spectrophotometer (AAS) at the respective wavelength of each metal using each metal respective hollow cathode lamp to atomise.

3.13.4.2 Determination of Serum retinol in blood

A well-homogenized sample of 0.5mL was weighed up into a 250mL Quartz round flask (QRF). 25mL methanol and 10mL 50% KOH was added for stability and suspended in a water bath set at 100^{o}C connected to a condenser (cold finger type) for 30 mins to reflux. The QRF mixture was then cooled in ice inside a darkroom for 1hr. The whole mixture in QRF was relocated to a 250mL volumetric flask and washed with 3:1 methanol/H_2O mixture and made up to 250mL mark. The flask was rotated up and down to ensure uniform mixing. The volumetric flask was put in the dark overnight. 20ml supernatant of the above was pipetted into a centrifuge tube and 20mL petroleum ether added and shakes for 1min. This mixture was centrifuged for 30min in a Gallenkamp centrifuge. 2ml of the supernatant from the centrifuge tube was pipetted into a 20mL tube and 1mL of chloroform added. 10mL of carr-price reagent (20% antimony chloride dissolved in chloroform with acetone). USP reference standard solution of trans-retinyl acetate which is equivalent to 30mg retinol was used as stock and working standard of range 0-5ug/mL was made from the stock. The working standard was treated like the sample above. The absorbance of standard and sample was measured on a Cecil 2483 spectrophotometer at a 430nm wavelength.

Vitamin A unit/100mL as retinol is evaluated as:

= [(Absorbance of standard) ÷ (Absorbance of the sample)] **x** [Conc. of standard]

3.13.5 Histological Preparation of Tissues

Liver, spleen, intestine and kidney were immediately fixed in 10% formalin, dehydrated in different alcohol concentrations; 70% alcohol for 2hrs, 95% alcohol for 2hrs, 100% alcohol for 2hrs. Serial sections were cut using rotary microtone at 5µm, satisfactory ribbons were selected from the water bath (50-55 ^{O}C) with microscope slides coated on one side with egg albumin as an adhesive and slides oven-dried. Each section was de-paraffinized in xylene for 1min before immersion in absolute alcohol and subsequently in plunging grades of alcohol for about 30secs each for hydration. Slides were rinsed in water, immersed in alcoholic hematoxylin for about 18mins, again rinsed in water, differentiated in 1% acid-alcohol, suspended running tap water to blue and later counterstained in alcoholic eosin for 30secs, rinsed in water for few seconds, before immersionfor 30sec each for dehydration of the preparations. Preparations were alcohol cleared by plunging in xylene for 1min, cleaned, blotted and mounted with DPX and coverslip, for examination under the microscope. Photomicrographs were taken at a magnification of x40, x100 and x400.

3.14.0 Sensory evaluationof Vegetable Soups

The vegetable soups sensory evaluation was carried out at the Department of Human Nutrition and Dietetics sensory evaluation laboratory, University of Ibadan. The standardized Vegetable soups based on the modified (NFSMI, 2010) method were assessed for their acceptability using 30 untrained panellists drawn within the University community who had eaten or known *C. forda lava* before and were willing to participate. The soups samples were rated on a 9-point hedonic scale in which the degree to which a product is relished was expressed as like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (3), dislike very much (2), dislike extremely (1). The panellists were required to observe the sample, taste and score based on colour, taste, aroma, texture and overall acceptability. The panellists were provided with water to rinse their mouths in between

sample evaluation and were instructed to rinse their mouth with water before tasting another sample.

3.15.0 Microbiological Analysis of *Cirina forda* Samples

Procedure

Laboratory workbenches were sterilized using cotton wool dipped in 70% ethanol.The culture media in this study were weighed out using the manufacturer's instructions and autoclaved at 121°C for 15mins.The samples were grounded into powder using an alcohol-sterilized laboratory pestle and mortar. The method of Harrigan and MacCance, (1976) was used to enumerate the bacterial load in the samples. One gramme of powdered *Cirina forda* was weighed into 10ml of sterile peptone water to serve as the stock.

Ten-fold serial dilution was carried out on the sample using peptone water as the diluent. Aliquots (1ml) of the chosen dilutions were plated out on the Nutrient agar (for the Heterotrophic bacterial count) and Potato Dextrose agar (for the fungal count). The set up was allowed to set and incubated at 35- 37°C for 24- 48 hours in the case of nutrient agar and at room temperature for 72-96 hours for the Potato Dextrose agar plates. The plates were observed after the incubation period and observations were noted and recorded.

The characterization of the fungi was carried out using the morphological and microscopic technique (Lactophenol Blue staining technique).

3.16.0 Statistical Analysis

The data from chemical analyses of the *Cirina forda*, and vegetable samples were expressed as mean and standard deviation of triplicate determinations. Test for significant differences was carried out using independent t-test, while the data obtained from the sensory evaluation was presented as means of 30 panellists' assessment and analysed using one – way ANOVA. Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., USA was used for the analyses, and the level of significance was set at $p<0.05$. One way ANOVA was used to examine variation in proximate and mineral composition, amino-acid, fatty acid, hematological analysis and serum chemistry tests, vitamin and anti-nutrient content, of *C. forda*samples obtain from different location (Ghana, Nigeria and Burkina Faso at level of significance, ($P<0.5$) and Dunnett's posthoc test.

CHAPTER FOUR

RESULTS

Specific Objective 1

To assessthe consumption pattern and knowledge of nutritional benefits of *Cirina forda* larva in the selected locations

4.1 Socio-demographic characteristics of the respondents

The result of the socio-demographic characteristics of respondents in the survey was shown in Tables 4.1. Mean age of the respondents was 46.3±16.4years, 38.0%, 35,5%,23.5% and 2.8% were within the age range of 20-39, 40-59, 60-79 and 80-99 years, respectively. More than half (54.8%)of the respondents were females, 43.2% were traders, 20.1% were artisan while 15.3% were farmers, the majority (61.4%) being females. Almost all (99.5%) the respondents were Yoruba, 69.7% practised Islamic religion while 28.5% were Christians. Also, (37.8%) respondents reported no formal education, but 25.3% reported secondary education, one-quarter (25.1%) of the respondents had primary education while 11.8% had tertiary education. The majority (81.1%) of the respondents had household size within the range of 0 – 9 members. Household income of 61.6% of the respondents was less than ₦20,000 per month, 26.9% earned between ₦20000 and ₦39,000, 6.6% earned between ₦40000-₦59000 while only 5.0% earned ₦60,000 and above (Table 4.1). More than half (56.4%) of the respondents were from Saki East while 43.6% were from Iseyin LGAs. Most (80.7%) of the respondents were married, while 4.8%, 0.8%, 3.4% and 10.4% were single, divorced, separated and widowed, respectively.

Table 4.1: Socio-demographic characteristics of the respondents

Variable	Total N (%)	Male n (%)	Female n (%)
< 20	1 (0.2)	0 (0.0)	1 (0.3)
20-39	191 (38.0)	65 (31.1)	126 (43.0)
40- 59	178 (35.5)	85 (40.7)	93 (31.7)
60-79	118 (23.5)	51 (24.4)	67 (22.9)
80-99	14 (2.8)	8 (3.8)	6 (2.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Mean age = 46.3±16.4			
Sex			
Male	209 (41.6)	209 (100.0)	0 (0.0)
Female	293 (58.4)	0 (0.0)	293 (100.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Occupation			
Civil Servant	25 (5.0)	15 (7.2)	10 (3.4)
Trader	217 (43.2)	37 (17.2)	180 (61.4)
Artisan	101 (20.1)	48 (23.0)	53 (18.1)
Farmer	77 (15.3)	49 (23.4)	28 (9.6)
Others (specify)	82 (16.3)	60 (28.7)	22 (7.5)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Ethnic group			
Yoruba	501 (99.8)	208 (99.5)	293 (100.0)
Hausa	1 (0.2)	1 (0.5)	0 (0.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Religion			
Christianity	143 (28.5)	52 (24.9)	91 (31.1)
Islam	350 (69.7)	151 (72.2)	199 (67.9)
Traditional	9 (1.8)	6 (2.9)	3 (1.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Level of Education			
Tertiary	59 (11.8)	29 (13.9)	30 (10.2)
Secondary	127 (25.3)	49 (23.4)	78 (26.6)
Primary	126 (25.1)	63 (30.1)	63 (21.5)
No formal Education	190 (37.8)	68 (32.5)	122 (41.6)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
0-9	407 (81.1)	159 (76.1)	248 (84.6)
10-19	89 (17.7)	46 (22.0)	43 (14.7)
20-29	6 (1.2)	4 (1.9)	2 (0.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

Table 4.1: Socio-demographic characteristics of the respondents (Cont'd)

Variable	Total N (%)	Male n (%)	Female n (%)
Household Income (₦)			
< 20,000	309 (61.6)	103 (49.3)	206 (70.3)
20,000-39,000	135 (26.9)	72 (34.4)	63 (21.5)
40,000-59,000	33 (6.6)	20 (9.6)	13 (4.4)
60,000-79,000	7 (1.4)	3 (1.4)	4 (1.4)
80,000-99,000	9 (1.8)	5 (2.4)	4 (1.4)
100,000 and above	9 (1.8)	6 (2.9)	3 (1.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Ward			
SE6	46 (9.2)	25 (12.0)	21 (7.2)
SE5	47 (9.4)	23 (11.0)	24 (8.2)
SE4	45 (9.0)	13 (6.2)	32 (10.9)
SE3	36 (7.2)	20 (9.6)	16 (5.5)
SE2	49 (9.8)	20 (9.6)	29 (9.9)
IS3	60 (12.0)	28 (13.4)	32 (10.9)
IS1	60 (12.0)	25 (12.0)	35 (11.9)
IS6	60 (12.0)	25 (12.0)	35 (11.9)
IS5	40 (8.0)	8 (3.8)	32 (10.9)
IS4	19 (3.8)	8 (3.8)	11 (3.8)
IS7	40 (8.0)	14 (6.7)	26 (8.9)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
LGA			
Saki East	283 (56.4)	121(57.9)	162 (55.3)
Iseyin	219 (43.6)	88 (42.1)	131 (44.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Marital Status			
Single	24 (4.8)	8 (3.8)	16 (0.5)
Married	405 (80.7)	184 (88.0)	221 (75.4)
Divorced	4 (0.8)	1 (0.5)	3 (1.0)
Separated	17 (3.4)	4 (1.9)	13 (4.4)
Widowed	52 (10.4)	12 (5.7)	40 (13.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

4.2 Pattern and frequency of *Cirina forda* consumption by the respondents

In Table 4.2, 99.8%of the respondents were aware of the existence of *Cirina forda*, 78.7% referred to it as "Monimoni" while 17.5% refer to it as "Ikanni" in the local language. All respondents (100%)reportedly consumed they had consumed*Cirina forda* before, 93.2% started its' consumption since childhood, 81.9% had consumed it within the last 2 years, while 18.1% had not.Approximately, 60.0% of those who did not consume it in the past two years attributed their non-consumption to scarcity, while 14.4% and 8.9% attributed it to marriage and civilization/scarcity respectively. About seventy-eight percent (78.9%) of respondents often consume *Cirina forda*, 45.9% consumed it weekly, while 8.2% consumed it daily.

Table 4.2, 87.1% reported that "all" members of their household consume *Cirina forda*, 35.4% and 35.9% of the respondents consumed it in the form of roasted/dried, and boiled/fried respectively, 19.9% consumed it in fried form, while 74.9% preferred its consumption as a condiment in the soup. About 60.0% of respondents preferred *Cirina forda* as a condiment in "Efo" while 30.1% and 41.6% preferred it with tomato sauce, and tomato sauce, "efo" and "egusi" respectively. A majority (64.3%) reported that there is a difference in the consumption rate of *Cirina forda* in the past and the present, while 21.8% and 25.9% attributed it to civilization; civilization, seasonality and price respectively; while 5.4%, 5.2% and 1.6% attributed it to income; seasonality; and death/poison respectively.Close to 22.9% and 24.9% reported that other edible insects that are consumed in their locality were "Ire" (Crickets), and "Esunsun" (Winged termites) respectively.

Table 4.2: Pattern and frequency of *Cirina forda* consumption by the respondents

Variable	Total N (%)	Male n (%)	Female n (%)
Knowledge of*Cirina forda* (Monimoni)			
Yes	501 (99.8)	209 (100.0)	292 (99.7)
No	1 (0.2)	0 (0.0)	1 (0.3)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Local name for *Cirina forda*			
Monimoni	395 (78.7)	172 (82.3)	223 (76.1)
Ikanni	88 (17.5)	32 (15.3)	56 (19.1)
Others (specify)	19 (3.8)	5 (2.4)	14 (4.8)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Prior consumption of *Cirina forda*			
Yes	502 (100.0)	209 (100.0)	293 (100.0)
No	0 (0.0)	0 (0.0)	0 (0.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
The outset of consumption of *Cirina forda*			
Since childhood	468 (93.2)	200 (95.7)	268 (91.5)
Adolescent	22 (4.4)	7 (3.3)	15 (5.1)
Adulthood	10 (2.0)	1 (0.5)	9 (3.1)
Others (Specify)	2 (0.4)	1 (0.5)	1 (0.3)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Consumption of *Cirina forda in the last two years*			
Yes	411 (81.9)	171 (81.8)	240 (81.9)
No	91 (18.1)	38 (18.2)	53 (18.1)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
If no Reason for not			
Marriage	13 (14.4)	8 (21.6)	5 (9.4)
Civilization	8 (1.6)	3 (8.1)	5 (9.4)
Scarcity	54 (60.0)	20 (54.1)	34 (64.2)
Civilization & Scarcity	8 (8.9)	4 (10.8)	4 (7.5)
Poisonous species	7 (7.8)	2 (5.4)	5 (9.4)
Total	**90 (100.0)**	**37 (100.0)**	**53 (100.0)**
Consumption of*Cirina forda*			
Yes	396 (78.9)	161 (77.0)	235 (80.2)
No	106 (21.1)	48 (23.0)	58 (19.8)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Frequency of Consumption			
Daily	41 (8.2)	20 (9.6)	21 (7.2)
Weekly	229 (45.9)	95 (45.5)	134 (45.7)
Monthly	105 (20.9)	40 (19.1)	65 (22.2)
Rarely	127 (25.3)	54 (25.8)	73 (24.9)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

Table 4.2: Consumption pattern and frequency of *Cirina forda* by the respondents(Continued)

Variable	Total N (%)	Male n (%)	Female n (%)
Other members of the household that had consumed*Cirina forda*			
All	437 (87.1)	188 (90.0)	249 (85.0)
Husband	11 (2.2)	0 (0.0)	11 (3.8)
Wife	10 (2.0)	10 (4.1)	0 (0.0)
Children	12 (2.4)	3 (1.4)	9 (3.1)
None	32 (6.4)	8 (3.8)	24 (8.2)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Frequency of the Consumption of thefamily member			
Daily	41 (8.2)	19 (9.1)	22 (7.5)
Weekly	225 (44.8)	95 (45.5)	130 (44.5)
Monthly	99 (19.8)	40 (19.1)	59 (20.2)
Rarely	106 (21.2)	47 (22.5)	59 (20.2)
Never	30 (6.0)	8 (3.8)	22 (7.5)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
The prefered form of*Cirina forda*consumption			
Roasted / dried	173 (35.4)	61 (29.2)	112 (38.2)
Boiled	46 (9.2)	17 (8.1)	29 (9.9)
Powdered	3 (0.6)	1 (0.5)	2 (0.7)
Fried	100 (19.9)	39 (18.7)	61 (20.8)
Boiled and fried	180 (35.9)	91 (43.5)	89 (30.4)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Quantity			
< 5	3 (0.6)	1 (0.5)	2 (0.7)
5-10	81 (16.1)	24 (11.5)	57 (19.5)
11-20	158 (31.5)	107 (36.5)	107 (36.5)
>20	260 (51.8)	127 (43.3)	127 (43.3)
Total	**502 (100.0)**	**293 (100.0)**	**293 (100.0)**
Preferred consumption form			
Pack	6 (1.2)	1 (0.5)	5 (1.7)
Spice in food	23 (4.6)	11 (5.3)	12 (4.1)
Condiment in soup	376 (74.9)	144 (68.9)	232 (79.2)
Bottled / fried	6 (1.2)	3 (1.4)	3 (1.0)
Others (Specify)	91 (18.1)	50 (23.9)	41 (14.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

Table 4.2: Consumption pattern and frequency of *Cirina forda* by therespondents (cont'd)

Variable	Total N (%)	Male n (%)	Female n (%)
Preferencefor*Cirina forda* as a condiment in soups			
Snack/Spice	31 (6.2)	14 (6.7)	17 (5.8)
Tomato sauce	151 (30.1)	51 (24.4)	100 (34.1)
"Efo"	30 (60.0)	17 (8.1)	13 (4.4)
"Egusi"	32 (6.4)	17 (8.1)	15 (5.1)
Tomato sauce, "Efo"and"Egusi"	209 (41.6)	85 (40.7)	124 (42.3)
All	44 (8.8)	22 (10.5)	22 (7.5)
Others	5 (1.0)	3 (1.4)	2 (0.7)
Total	502 (100.0)	209 (100.0)	293 (100.0)
Past and Present different in consumption rate			
Yes	323 (64.3)	128 (61.2)	195 (66.6)
No	157 (31.3)	72 (34.4)	85 (29.0)
Don't know	22 (4.4)	9 (4.3)	13 (4.4)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Reason for the difference			
Income	27 (5.4)	10 (4.8)	17 (5.8)
Civilization	109 (21.8)	39 (18.8)	70 (24.1)
Seasonality	26 (5.2)	15 (7.2)	11 (3.8)
Education	3 (0.6)	2 (1.0)	1 (0.3)
Price	6 (1.2)	4 (1.9)	2 (0.7)
Civilization & seasonality	12 (2.4)	5 (2.4)	7 (2.4)
Civilization, seasonality & price	130 (25.9)	49 (23.6)	81 (27.8)
Death/poison	8 (1.6)	4 (1.9)	4 (1.4)
No response	182 (35.7)	81 (38.5)	100 (33.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Other popular edible insects in the locality			
Esunsun	34 (6.8)	7 (3.3)	27 (9.2)
Igango	28 (5.6)	14 (6.7)	14 (4.8)
Ire	115 (22.9)	57 (27.3)	58 (19.8)
Esunsun& Ire	125 (24.9)	60 (28.7)	65 (22.2)
All	31 (6.2)	16 (7.7)	15 (5.1)
I don't know	169 (33.7)	55 (26.3)	114 (38.9)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

4.3 Availability and accessibility of *Cirina forda* to the respondents

Table 4.3 showed 82.4% of the respondents reportedly confirmed that *Cirina forda* was very popular in their locality while 1.4% and 0.6% reported that *Cirina forda* was rarely known and not known at all respectively. All (100%) respondents reported *Cirina forda* to be acceptable in their locality. The majority (79.5%) of them acknowledged that *Cirina forda* were not available all year round in their locality, 92.4% reported that it is mainly available during the rainy/wet season, while only 3.2% reported its availability during the dry season. Most (98.4%) of respondents reported that *Cirina forda* were usually preserved/stored in dried form; 34.7% reported that*Cirina forda*can be preserved for between one and three months while 19.3% reported that it can be preserved/stored for more than 12 months. More than half (56.4%) of the respondents mentioned that they purchased *Cirina forda* using milk tin cup as a way/medium of measurement while 4.2% and 23.5% used tomato tin and Mudu/Kongo for measurement respectively.

Table 4.3: Availability and accessibility of *Cirina forda* of the respondents

Variable	Total N (%)	Male n (%)	Female n (%)
The popularity of *Cirina forda*in the locality			
Very popular	413 (82.4)	181 (86.6)	232 (79.2)
Popular	78 (15.6)	26 (12.5)	52 (17.7)
Rarely known	7 (1.4)	0 (0.0)	7 (2.4)
Not known at all	4 (0.6)	2 (0.5)	2 (0.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Acceptability of *Cirina forda* in the locality			
Very acceptable	370 (73.3)	164 (78.5)	206 (70.3)
Acceptable	132 (26.3)	45 (21.5)	87 (29.7)
Total	502 (100.0)	209 (100.0)	293 (100.0)
Availability of *Cirinaforda* all year round in the locality			
Yes	82 (16.3)	45 (21.5)	37 (12.6)
No	399 (79.5)	160 (76.6)	239 (81.6)
I don't know	21 (4.2)	4 (1.9)	18 (5.8)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Season of the year of *Cirinaforda* Availability			
Raining (Wet)	379 (92.4)	158 (75.6)	221 (75.4)
Dry	16 (3.2)	43 (20.6)	64 (21.8)
Don't know	22 (4.4)	8 (3.8)	8 (2.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Storage Status of *Cirinaforda*			
Yes	464 (92.4)	194 (92.8)	270 (92.2)
No	16 (3.2)	6 (2.9)	10 (3.4)
I don't know	22 (4.4)	9 (4.3)	13 (4.4)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Form ofpreservation			
Dried	494 (98.4)	206 (98.6)	288 (98.3)
Wet	2 (0.4)	1 (0.5)	1 (0.3)
Others (Specify)	6 (1.2)	2 (1.0)	4 (1.4)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Shelf-life of *Cirina forda*			
< 1	62 (12.4)	21 (10.0)	41 (14.0)
1-3	174 (34.7)	53 (25.4)	121 (41.3)
4-6	94 (18.7)	48 (23.0)	46 (15.7)
7-12	75 (14.9)	35 (16.7)	40 (13.7)
>12	97 (19.3)	52 (24.9)	45 (15.4)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

Table 4.3: Availability and accessibility of *Cirina forda* of the respondents (cont'd)

Variable	Total N (%)	Male n (%)	Female n (%)
Availability of *Cirina forda*in the marketall year-round			
Yes	206 (41.1)	90 (43.3)	116 (39.6)
No	265 (52.9)	105 (50.5)	160 (54.6)
Don't know	31 (6.0)	14 (6.3)	17 (5.8)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Form of purchase of *Cirina forda*in the market			
Tomato tin	21 (4.2)	7 (3.3)	14 (4.8)
Milk tin	283 (56.4)	101 (48.3)	182 (62.1)
Rubber	73 (14.5)	40 (19.1)	33 (11.3)
Mudu/Kongo	118 (23.5)	57 (27.3)	61 (20.8)
Others (Specify)	7 (1.4)	4 (1.9)	3 (1.0)
Total	**502 (100.0)**	**209 (100.0)**	**209 (100.0)**
Period for consuming the purchased quantity			
0-9 days	472 (94.0)	199 (95.2)	273 (93.2)
10-19 days	24 (4.8)	9 (4.3)	15 (5.1)
20-29 days	4 (0.8)	1 (0.5)	3 (1.0)
30-39 days	1 (0.2)	0 (0.0)	1 (0.3)
>39 days	1 (0.2)	0 (0.0)	1 (0.3)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Availability in the market			
Yes	458 (91.2)	198 (94.7)	260 (88.7)
No	37 (7.4)	9 (4.3)	28 (9.6)
I don't know	7 (1.4)	2 (1.0)	5 (1.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Cost of *Cirina forda*			
0-9,000	501 (99.8)	208 (99.5)	293 (100.0)
10,000 and above	1 (0.2)	1 (0.5)	0 (0.0)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**

4.4 Respondents' awareness of the benefits of *Cirina forda* consumption

In Table 4.4, 29.7% reported that they consumed *Cirina forda* as a result of food habit and custom,17.0%, 17.2%, and 11.6% reported their consumption to be due to flavour/taste, nutrition/health benefits, food habits/economic benefits, respectively. More than half (58.9%) of the respondents were not awareof the nutrition and health benefits of *Cirina forda*, 29.7% reported that the insect larvae provides energy, proteins and vitamins, while 7.8%, 1.2% and 2.4% reported that it helps in blood circulation, clear vision, and bowel movement respectively. More than one-third of the respondents (38.1%) reportedly consume larvae because of the perceivedbenefits of *Cirina forda*which influenced their consumption, while 12.8% did not know if the benefits of *Cirina forda* influenced their consumption pattern. Most respondents (96.0%) stated they did not know any health/toxicity-related issue as regards *Cirina forda* consumption. However, 1.8% mentions that there is a health hazard or toxic effect on consumption of the larvae of which 37.5% attributed flatulence to the consumption of *Cirina forda* and 25.0% each reported death and cough respectively as the health-related issues upon the consumption of *Cirina forda*

4.5 Limitation and restriction to *Cirina forda* consumption by the Respondents

In Table 4.5, almost all the respondents (99.8%) reported that religion does not affect the consumption of *Cirina forda,* 99.0% mentioned that there is no traditional belief or myth attached to it. The respondent agreed that availability (56.1%), age (99.0%) and cost does not affect insect larvae consumption, but food habit or custom (64.5%) was the general opinion of the people as regards *Cirina forda* consumption in their locality. Almostforty per cent (39.9%) of respondents preferred *Cirina forda* consumption to meat, fish or eggs.

Table 4.4: Respondents' awareness of the benefits of *Cirina forda* consumption

Variable	Total N (%)	Male n (%)	Female n (%)
Reason for the consumption of *Cirinaforda*			
Flavor and taste	85 (17.0)	30 (14.4)	55 (18.8)
Food habit/custom	149 (29.7)	63 (30.1)	86 (29.5)
Nutrition/health benefit	86 (17.2)	45 (21.5)	41 (14.0)
Food habit/Economical	58 (11.6)	26 (12.4)	7 (2.4)
All except nutritional benefits	35 (7.0)	14 (6.7)	32 (11.0)
All of the above	75 (15.0)	25 (12.0)	21 (7.2)
Others (Specify)	14 (2.6)	6 (2.9)	51 (17.1)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Nutrition and Health benefit of *Cirina forda*			
Helps in blood circulation	39 (7.8)	23 (11.0)	16 (5.5)
Clear vision	6 (1.2)	4 (1.9)	2 (0.7)
Bowel movement	12 (2.4)	9 (4.3)	3 (1.0)
Don't know	295 (58.9)	115 (55.0)	180 (61.6)
Gives energy, protein & vitamins	150 (29.7)	58 (27.8)	92 (31.1)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Benefit effects on consumption of *Cirina forda*			
Yes	191 (38.1)	88 (42.1)	103 (35.3)
No	246 (49.1)	100 (47.8)	146 (50.0)
I don't know	65 (12.8)	21 (10.0)	44 (14.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Health-related issue associated with *Cirina forda* consumption			
Yes	9 (1.8)	2 (1.0)	7 (2.4)
No	481 (96.0)	204 (97.6)	277 (94.5)
I don't know	12 (2.2)	3 (1.4)	9 (2.7)
Total	**502 (100.0)**	**209 (100.0)**	**293 (100.0)**
Health-related issues			
Flatulence	3 (37.5)	2 (100.0)	1 (16.7)
Itchy skin	1 (12.5)	0 (0.0)	1 (16.7)
Death	2 (25.0)	0 (0.0)	2 (33.3)
Cough	2 (25.0)	0 (0.0)	2 (33.3)
Total	**8 (100.0)**	**2 (100.0)**	**6 (100.0)**

Table 4.5: Limitation and restriction to *Cirina forda* consumption by the Respondents

Variable	Total N (%)	Male n (%)	Female n (%)
Effect of religious belief on consumption of *Cirina forda*			
Yes	1 (0.2)	1 (0.3)	0 (0.0)
No	500 (99.8)	209 (99.7)	292 (100.0)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
Knowledge of traditional issues associated with *Cirina forda consumption*			
Yes	5 (1.0)	1 (0.5)	4 (1.4)
No	496 (99.0)	208 (99.5)	288 (98.6)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
Consumption-based on Availability			
Yes	220 (43.9)	82 (39.2)	138 (47.1)
No	281 (56.1)	127 (60.8)	154 (52.7)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
Effect of Age			
Yes	5 (1.0)	1 (1.4)	2 (0.7)
No	496 (99.0)	206 (98.6)	290 (99.3)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
Effect of cost			
Yes	3 (0.6)	2 (1.0)	1 (0.3)
No	498 (99.4)	207 (99.0)	291 (99.7)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
If yes, why?			
Too expensive	3 (100.0)	2 (100.0)	1 (100.0)
Perception of people about *Cirina forda*			
Nutritious	47 (9.4)	22 (10.5)	25 (8.6)
Economical	58 (11.6)	27 (12.9)	31 (10.6)
Food habit/custom	323 (64.5)	136 (65.1)	187 (64.0)
Don't know	37 (7.4)	8 (3.8)	29 (9.9)
Civilization/fading interest	35 (7.0)	16 (7.7)	19 (6.5)
Death /disease	1 (0.2)	0 (0.0)	1 (0.3)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**

Table 4.5: Limitation and restriction to the consumption of *Cirina forda* of the Respondents (Cont'd)

Variable	Total N (%)	Male n (%)	Female n (%)
Preference of Cirina forda to meat, fish or eggs			
Yes	200 (39.9)	80 (38.3)	120 (41.1)
No	277 (55.3)	121 (57.9)	156 (53.4)
Indifference	24 (4.8)	8 (3.8)	16 (5.5)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**
Reason			
Fleshy/boneless	24 (4.8)	7 (3.3)	17 (5.8)
Personal preference	157 (31.3)	67 (32.1)	90 (30.8)
Economical	32 (6.4)	12 (5.7)	20 (6.8)
Nutrition /health benefits	60 (12.0)	29 (13.9)	31 (10.6)
Taste/flavour	60 (12.0)	21 (10.0)	39 (13.4)
Indifference	31 (6.2)	11 (5.3)	20 (6.8)
Others (specify)	137 (27.3)	62 (29.7)	75 (25.7)
Total	**501 (100.0)**	**209 (100.0)**	**292 (100.0)**

Specific Objective 2

To determine and compare the nutrient and anti-nutrient compositionof *Cirina forda*larvae from selected locations

4.6 Proximate nutrient composition of *Cirina forda* larva samples

Table 4.6 showedthat the proximate content of the *Cirina forda* larva samples from Saki (Nigeria), Ghana, and Bobo Diolasso (Burkina Faso). There were significant differences ($p<0.05$) in the nutrient composition of the various samples. Saki sample was highest in moisture (4.01%), crude fibre (0.86%) and carbohydrate content (23.92%), Ghana sample was highest in fat (17.21%), ash gross energy(458.63kcal/), and dietary fibre content (9.12%), while Burkina Faso was highest in crude protein content (54.38%0. Generally from the result of this study, the dry *Cirina forda*larva contained a high amount of protein, fat, gross energy and dietary fibre, but low in moisture with the mean value of moisture content as 3.7±0.41, crude protein 53.24±1.26, crude fat 16.90±0.27, ash 0.72±0.16, carbohydrates 22.44±1.28, dietary fibre 8.79±0.37 and gross energy 452.089kcal/100g *Cirina forda*

4.7 Mineral composition of *Cirina forda* larva samples

Table 4.7 showed the mineral contents of the various samples of the *Cirina forda*differed significantly ($p<0.05$) in calcium, phosphorus, magnesium and iron. Ghana sample has the highest values for all themicro-nutrients (K:649; Na:309; Ca:281; P:163; Fe:9.24; Zn:1.51 mg/100g, and Se:403.81µg/100g) except for magnesium where there was no significant difference ($p>0.05$) across the three samples. Saki samples rank second highest in the mineral values except in selenium value where Burkina Faso sample ranks the second-highest (Se:401.96µg/100g) after Ghana sample. The mean mineral content for the samples used in the study were sodium 296.89±0.11, potassium 648.33±0.40, calcium 270.56±0.96, phosphorus 286.45±0.77, iron 8.91±0.34, copper 0.34±0.05, zinc 1.43±0.07, manganese 0.01±0.00 and selenium 400.06±0.50mg/100g *Cirina forda*.

4.8 Vitamin composition of *Cirina forda* larva samples

Table 4.8 showed the vitamin content of the *Cirina forda* samples and there weresignificant differences($p<0.05$) in vitamins A, Bs' and C content of the samples. Ghana samples recorded the highest values also for vitamins A, B_2, B_{12}, C and E as 0.39, 0.44, 3.49, 19.33 and 2.05mg/100g respectively. Saki sample was highest in B1 and B3, while Burkina Faso sample recorded highest for $B_{12,}$ 3.53mg/100g. The result showed the *Cirina forda*larva has substantial values of Vitamins C, B_{12} and E

4.9 Antinutrient content in *Cirina forda* Larva

The anti-nutrient content of the larva samplesis shown in Table 4.9. *Cirina forda*samples were not detectable for phytate, tannin and saponin and very lowfor oxalate with no significant difference ($p>0.05$). However, trypsin inhibitor showeda significant difference($P<0.05$) in which Saki larva samplehas the highest value comparedto Ghana and Burkina Faso samples.

4.10 Amino acids composition of *Cirina forda* larva

Table 4.11 showed the *Cirina larva* samples contained appreciable values at ($P<0.05$) for all the essential amino acids, EAAs with lysine as most abundant with glutamic acid, threonine, aspartic acid, leucine, glycine,isoleucine, tyrosine and phenylalanine in moderate amount in the larva among others. Hence, the samples contained protein content of high quality.

4.11 Fatty acid compositionof *Cirina forda*larva

In Table 4.13, the larva samples showed that they were rich in both saturated and unsaturated fatty acids, with the polyunsaturated fatty acids being the highest in *Cirina forda*samples. The same patterns were recorded for the three samples of the different locations with linolenic have the predominate concentration of overall mean of 32,93%, followed by stearic, 26.9%, palmitic, 16.3%, and oleic, 11.3% respectively at level of significance ($P<0.05$).

Table 4.6: Proximate composition of *Cirina forda* larva (g/100g)

Parameter	Sample S, *C. forda*Saki	Sample G, *C. forda* Ghana	Sample BF, *C. forda* Burkina Faso	Overall Mean
Moisture	4.01 ± 0.02^{a}	3.29 ± 0.02^{b}	3.98 ± 0.03^{a}	3.76 ± 0.41
Protein	51.88 ± 0.10^{a}	53.45 ± 0.16^{b}	54.38 ± 0.01^{c}	53.24 ± 1.26
Fat	16.69 ± 0.03^{a}	17.21 ± 0.03^{b}	16.81 ± 0.03^{c}	16.90 ± 0.27
Crude Fibre	0.86 ± 0.02^{a}	0.75 ± 0.02^{b}	0.55 ± 0.02^{c}	0.72 ± 0.16
Ash	2.51 ± 0.03^{a}	2.71 ± 0.02^{b}	2.58 ± 0.03^{a}	2.6 ± 0.10
Carbohydrate	23.92 ± 0.03^{a}	21.68 ± 0.04^{b}	21.74 ± 0.07^{b}	22.44 ± 1.28
Gross Energy (kcal/)	442.03 ± 0.31^{a}	458.63 ± 0.31^{b}	456.27 ± 0.35^{c}	452.31 ± 0.89
Dietary Fibre	8.39 ± 0.03^{a}	9.12 ± 0.04^{b}	8.86 ± 0.02^{c}	8.79 ± 0.37

Parameters withsimilar alphabets along the same row are not significantly different ($p>0.05$). Values are the mean and standard deviation of three determinations (n=3).

Table 4.7: Mineral composition of *Cirina forda* larva (mg/100g)

Parameter	Sample S Saki, *C. forda*	Sample G Ghana, *C. forda*	Sample BF Burkina Faso, *C. forda*	Overall Mean
Sodium	291.33 ± 3.06[a]	309.33 ± 2.52[b]	290.00± 2.00[a]	296.89 ± 0.11
Potassium	627.0 ± 2.0[a]	649.33± 3.06[b]	623.67 ± 3.06[a]	648.33 ± 0.40
Calcium	268.67± 2.52[a]	281.00 ± 3.00[b]	262. 00± 3.00[c]	270.56 ± 0.96
Phosphorus	155.00 ± 2.00[a]	163.00 ± 2.00[b]	146.67 ± 1.53[c]	154.89 ± 0.82
Magnesium	286.67 ± 2.52[a]	294.00± 2.00[b]	278.67± 3.51[c]	286.45 ± 0.77
Iron	8.56 ± 0.02[a]	9.24± 0.02[b]	8.94 ± 0.02[c]	8.91 ± 0.34
Copper	0.29 ± 0.03[a]	0.39 ± 0.03[b]	0.33 ± 0.02[a]	0.34 ± .0.05
Zinc	1.39 ± 0.03[a]	1.51 ± 0.03[b]	1.39 ± 0.03[a]	1.43 ± 0.07
Manganese	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01± 0.00[a]	0.01 ± 0.00
Selenium (µg/)	394.41 ± 0.05[a]	403.81± 0.03[b]	401.96 ± 0.01[c]	400.06 ± 0.50

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Values are the mean and standard deviation of three determinations ($n=3$).

Table 4.8: Vitamin composition of *Cirina forda* larva (mg/100g)

Parameter	Sample S Saki, *C. forda*	Sample G Ghana, *C. forda*	Sample BF Burkina Faso, *C. forda*	Overall Mean
Vitamin A (µg/)	0.27 ± 0.02^{a}	0.39 ± 0.03^{b}	0.33 ± 0.02^{c}	0.33 ± 0.06
Vitamin B1	0.35 ± 0.02^{a}	0.26 ± 0.3^{b}	0.19 ± 0.02^{c}	0.27 ± 0.08
Vitamin B2	0.34 ± 0.03^{a}	0.44 ± 0.03^{b}	0.37 ± 0.01^{a}	0.38 ± 0.05
Vitamin B3	0.90 ± 0.03^{a}	0.70 ± 0.03^{b}	0.62 ± 0.03^{c}	0.74 ± 0.14
Vitamin B6	0.41 ± 0.03^{a}	0.52 ± 0.04^{b}	0.46 ± 0.02^{a}	0.46 ± 0.05
Vitamin B12	3.19 ± 0.02^{a}	3.49 ± 0.03^{b}	3.53 ± 0.02^{b}	3.40 ± 0.19
Vitamin C	18.27 ± 0.02^{a}	19.33 ± 0.02^{b}	19.24 ± 0.03^{c}	18.95 ± 0.59
Vitamin E (µg/)	1.91 ± 0.03^{a}	2.05 ± 0.03^{b}	1.89 ± 0.03^{a}	1.95 ± 0.09

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Values are means ± standard deviation of three determinations ($n = 3$)

Table 4.9 Anti-nutrient Composition of *Cirina forda* larva (mg/100g)

Parameter	Sample S Saki, *C. forda*	Sample G Ghana, *C. forda*	Sample BF Burkina Faso, *C. forda*	Overall Mean
Phytate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
Oxalate	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
Tannin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
Saponin	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
Trypsin Inhibitor	0.81 ± 0.03^{a}	0.67 ± 0.03^{b}	0.70 ± 0.03^{b}	0.73 ± 0.07

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Values are means ± standard deviation of three determinations ($n = 3$)

Table 4.10: Amino acids composition of *Cirina forda* larva

Amino Acids	Sample S (%) Saki, *C. forda*	Sample G (%) Ghana, *C. forda*	Sample BF (%) Burkina Faso, *C. forda*	Overall Mean
Alanine	3.65	3.71	3.72	3.69
Arginine	2.79	2.83	2.85	2.82
Aspartic Acid	7.61	7.67	7.65	7.64
Cysteine	1.78	1.85	1.87	1.83
Glutamic Acid	13.22	13.31	13.33	13.29
Glycine	5.69	5.75	5.77	5.74
Histidine	3.47	3.55	3.54	3.52
Isoleucine	5.34	5.39	5.37	5.37
Leucine	5.65	5.76	5.77	5.73
Lysine	19.82	19.97	19.96	19.92
Methionine	2.11	2.21	2.19	2.17
Phenylalanine	4.37	4.46	4.47	4.43
Proline	3.34	3.39	3.36	3.36
Threonine	8.03	8.11	8.09	8.08
Tryptophan	1.56	1.66	1.64	1.62
Tyrosine	5.03	5.12	5.10	5.08
Ornithine	0.12	0.15	0.14	0.14
Cystine	0.57	0.68	0.67	0.64
Serine	0.16	0.22	0.24	0.21
Valine	2.48	2.57	2.56	2.54

Table 4.11: Fatty acid Composition in *Cirina forda* larva

Fatty Acids	Sample S (%) Saki, *C. forda*	Sample G (%) Ghana. *C. forda*	Sample BF (%) Burkina Faso. *C. forda*	Overall Mean
Arachidonic	0.21	0.34	0.330.29	
Behenic	0.00	0.00	0.000.00	
Caproic	0.00	0.00	0.000.00	
Caprylic	0.00	0.00	0.000.00	
Capric	0.00	0.00	0.000.00	
Erucic	0.00	0.00	0.000.00	
Lauric	3.29	3.38	3.383.35	
Linoleic	6.85	7.08	7.077.00	
Linolenic	32.67	33.05	33.0632.93	
Lignoceric	0.00	0.00	0.00	0.00
Margaric	0.07	0.03	0.030.04	
Myristic	0.58	0.64	0.630.62	
Oleic	11.18	11.37	11.3511.30	
Palmitic	16.23	16.39	16.3816.33	
Palmitoleic	0.34	0.41	0.430.39	
Stearic	26.49	27.17	27.1626.94	

Specific Objective 3

To carry out the toxicological study on *Cirina forda*larva usingrat models.

4.12 Average supplemented feed intake of rats

The Average supplemented feed intake of rats is presented in figure 4.1 below. Rats fed on 20% *Cirina forda* diet had an average supplemented feed intake of 15g, those fed on 80% *Cirina forda* diet had an average intake of 14.75g, the control group (rat pellet feed) had an average intake of 14.5g in the preliminary phase of the experiment, while at the final phase, 20% *Cirina forda* diet and 80% *Cirina forda* diet groups had 14g each and the control group had an average feed intake of 13.5g.

4.13 Effect of dosage of *Cirina forda*liquid extract on body weight change and average feed intake

In table 4.15, The average feed ingestion of rats dosed with *Cirina forda*liquid extract during the first week of the experiment was 15g and that of the control was 12g while during the second week of the experiment, rats dosed with *Cirina forda*liquid extract and the control group had an average intake of 15g each. The average body weight change of rats dosed with *Cirina forda*liquid extract ranged from 103.00-138.00g while that of the control range from 107.33-138.67g.

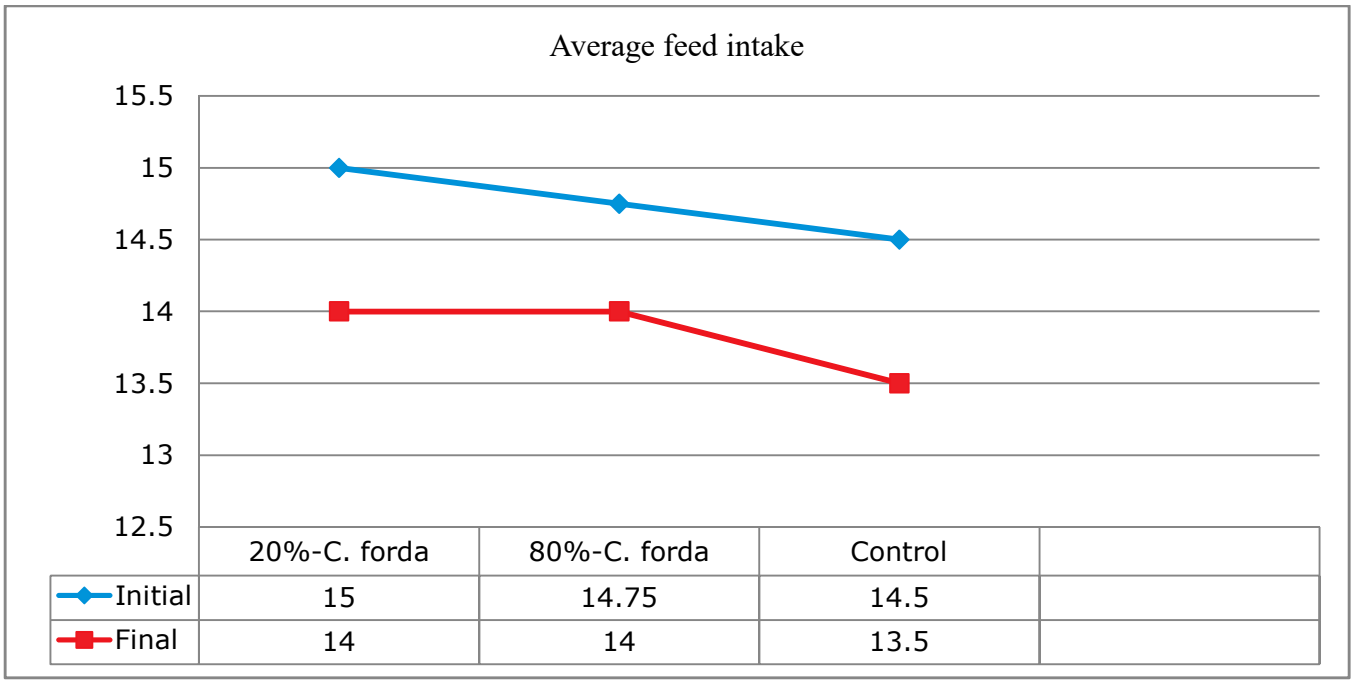


Fig. 4.1: Average supplemented feed intake of rats(g)

Table 4.12: Effect of the dosage of the *Cirina forda*liquid extract on body weight change and average feed intake

Groups	Weight (g)			Average Feed Intake	
	Initial	Final	Weight gain	1st Week	2nd Week
Control	107.33 ± 8.08[a]	138.67 ± 7.57[a]	31.33± 11.01[a]	12.00 ± 5.20	15.00 ±0.00
5000mg/kg	103.00 ± 8.72[a]	138.00±13.89[a]	35.00 ±22.61[a]	15.00 ±0.00	15.00 ±0.00

Values are Mean±SEM (n=4). *P<0.05 experimental vs control using one-way ANOVA and Dunnett's posthoc test.

4.14 Effect of supplemented feed on body weight change and relative organ weights

Table 4.16 showed an increment in the weight of rats in all the groups at the end of the experiment. First, the 20%-*Cirina forda* diet group had a weight gain of 26.50g, the 80%-*Cirina forda* diet group had a weight gain of 37.25g while the control group had a weight gain of 29.50g. The control supplemented feed group recorded the highest relative organ weight, relative organ weight in the brain, and second-highest weight for liver and kidney. The 20%-*Cirina forda* inclusion feed group has the highest relative organ weight for the Kidney and lowest relative organ weight for the liver and brain. The 80%-*Cirina forda* inclusion feed had the highest relative organ weight for the Liver, second highest for the brain and lowest for the kidney. There was no significant difference in the rats' relative organ weight across the different supplemented diets or feeds used except for relative organ weight of the rats fed at 80%-*Cirina forda* inclusion with a significant difference at ($P<0.05$).

4.15 Haematological values of animals fed with supplemented feeds for fourteen days

Haematological biomarkers of rats on the 14-day supplemental diets (Table 4.17) revealed no significant difference ($p>0.05$) compared to the control group.

4.16 Serum chemistry and nutrient values of animals fed with supplemented feeds for fourteen days

The Serum chemistry of the rats on supplemental diets (Table 4.18) reveals no significant difference compared to the control group, except for alkaline phosphatase (ALP) profile in the 80% *Cirina forda* diet that is rather on the high side.

The serum nutrient values of rats on the supplemental diet (Table 4.19) showed insignificantly higher values in the control group compared with the experimental groups ($p>0.05$).

Table 4.13: Effect of supplemented feed on body weight change and relative organ weights

Groups	Weight (g)			Relative organ weights		
	Initial	Final	Weight gained	Liver	Kidney	Brain
Control	130.8±13.42	160.30±11.08	29.50 ± 4.05	2.98 ± 0.27	0.63±0.05	0.93±0.16
20%	138.5 ± 5.87	165.00± 5.35	26.50 ± 1.56	2.57 ± 0.27	0.77±0.17	0.61±0.02
80%	130.5 ± 8.30	167.80± 9.83	37.25 ± 2.29	3.83 ± 0.38*	0.60±0.04	0.75±0.15

Values are Mean±SEM (n=4). *P<0.05 experimental vs control using one-way ANOVA and Dunnett's posthoc test.

Table 4.14: Haematological values of animals fed with supplemented feeds for fourteen days

Haematological indicators	Control	20%*C. forda* diet	80%*C. forda* diet
PCV (%)	46.75 ± 0.75	47.75 ± 0.85	45.25 ± 1.11
Hb (mg/dL)	15.60 ± 0.24	15.92 ± 0.34	14.90 ± 0.46
RBC (10^6/mL)	7.65 ± 0.16	7.90 ± 0.25	7.60 ± 0.21
Platelets (10^6/mL)	113.00 ± 14.71	105.00 ± 10.54	99.75 ± 12.43
WBC (10^3/mL)	34.5 ± 2.95	25.12 ± 3.74	33.75 ± 3.40
Lymphocytes (10^3/mL)	70.25 ± 1.65	71.50 ± 2.32	71.75 ± 1.75
Neutrophils (10^3/mL)	26.50 ± 2.06	25.00 ± 3.49	25.00 ± 1.47
Monocytes (10^3/mL)	1.75 ± 0.48	2.00 ± 0.41	1.75 ± 0.25
Eosinophils (10^3/mL)	1.50 ± 0.64	1.50 ± 1.19	1.50 ± 0.65

Values are Mean±SEM (n=4). *P<0.05 experimental vs control using one-way ANOVA and Dunnett's posthoc test

Table 4.15: Serum chemistry values of animals fed with supplemented feeds for fourteen days

Parameters	Control	20%*C. forda* diet	80%*C. forda* diet
Total protein (mg/mL)	6.98 ± 0.22	7.15 ± 0.23	6.75 ± 0.29
Albumin (mg/mL)	2.93 ± 0.18	2.95 ± 0.10	2.78 ± 0.16
Globulin (mg/mL)	3.90 ± 0.20	4.01 ± 0.26	3.98 ± 0.18
A.G (ratio)	0.73 ± 0.05	0.70 ± 0.04	0.70 ± 0.04
AST (U/L)	39.25 ± 1.60	40.00 ± 0.91	38.00 ± 1.47
ALT (U/L)	27.50 ± 2.26	28.25 ± 0.75	27.75 ± 0.85
ALP (U/L)	85.25 ± 4.50	93.25 ± 9.49	102.00 ± 2.48*
Blood Urea Nitrogen (BUN) (µmol/L)	16.18 ± 0.31	16.43 ± 0.31	16.05 ± 0.30
Creatinine (µmol/L)	0.55 ± 0.03	0.58 ± 0.03	0.55 ± 0.03
Total Bilirubin (µmol/L)	0.20 ± 0.01	0.25 ± 0.03	0.23 ± 0.03
Direct Bilirubin (µmol/L)	0.025 ± 0.003	0.030 ± 0.004	0.040 ± 0.001

Values are Mean±SEM (n=4). *P<0.05 experimental vs control using one-way ANOVA and Dunnett's posthoc test.

Table 4.16: Serum nutrient values of animals fed with Supplemented feeds

Parameters	Control (rat pellet feed)	20%*C. forda* diet	80%*C. forda* diet
Protein (g/dL)	6.77 ± 0.78	6.68±0.61	6.41±0.31
Calcium (mg/dL)	7.75 ± 1.81	7.43 ± 1.29	7.13±0.77
Zinc (mg/dL)	0.68 ± 0.27	0.65 ±1.00	0.61 ± 0.15
Iron (mg/dL)	11.76 ± 0.23	11.69±0.34	11.61±0.32
Cholesterol (mg/dL)	57.44 ± 2.46	51.28 ± 5.54	50.82±5.56
Vitamin A (R. EQ)	50.21±2.12	49.12±3.08	48.97±0.55

Values are Mean±SEM (n=4). *P<0.05 experimental vs control using one-way ANOVA and Dunnett's posthoc test

4.17 Urinalysis Results

The urinobilinogen was normal (with the absence of blood, nitrite, ketones and glucose) in all the groups. Bilirubin was found in moderate amounts in the control and 20% *Cirina forda* diet groups while it was found in severe amounts in 80% *Cirina forda* diet group (Table 4.20). Ascorbic acid was absent in the control group, while it was present in a mild amount in the 20% *Cirina forda* diet group and present in severe amounts in the 80% *Cirina forda* diet group. The control and the 20% *Cirina forda* diet groups had neutral P^H while the 80% *Cirina forda* diet group had slightly acidic P^H.

Table 4.17: Urinalysis Results

Parameters	Control	20% *C. forda* diet	80%*C. forda* diet
Blood	-VE	-VE	-VE
Urinobilinogen	Normal	Normal	Normal
Bilirubin	++	++	+++
Protein	30	30	30
Nitrite	-VE	-VE	-VE
Ketones	-VE	-VE	-VE
Ascorbic Acid	-VE	+	++
Glucose	-VE	-VE	-VE
pH	7	7	6

4.18 Proximate composition of *Cirina forda*powder and the feeds used

The proximate composition of the feed of animals used for toxicity study is shown in (Table 4.21). addition of *Cirina forda*powder to the feed at different inclusion level resulted in significant difference in proximate composition of the feeds ($p<0.05$). The protein, crude fat, ash, and gross energy content of the supplemented feeds (20% and 80% *Cirina forda* larva diets) were significantly higher than that of the 100% Commercial top-feed starter mash.

4.19 Mineral composition of *Cirina forda* powder and rats feed used

There were significant differences ($p<0.05$) in mineral content of the feeds (Table 4.22), but the similarity in values was observed in magnesium content of supplemented feeds (20% and 80% *Cirina forda* diets), zinc in the 100%-Commercial Top-feed starter mash and 80% *Cirina forda* larva feed, and selenium in the 100%-Commercial Top-feed starter mash and 20% *Cirina forda* feed.

4.20 Vitamin composition of *Cirina forda* powder and the feeds used (mg/100g)

In Table 4.23, thevitamin content of the supplemented feed differed significantly, with an increase in the value of vitamins A, E, K, and B_{12}, as the level of substitution of supplemented feeds increased (20% and 80% *Cirina forda* feed) compared with the 100% commercial top-feed starter mash feed. However, a significant decrease in the values of vitamins B_1, B_2, B_3, B_6, and C was observed in the supplemented feed as the level of*Cirina forda* supplementation increased($p<0.05$).

4.21 Antinutrient contentinthe feeds and *Cirina forda* powder

In Table 4.24, the feed and supplemented feed used were very low in the antinutrients studied. Oxalates and tannins were undetectable at the level of determination. The values of phytates for samples A and D, and B and C were not significantly different ($p>0.05$), while trypsin inhibitor significantly decreased in all the feeds with no significant difference in oxalate profiles of all feeds as well as phytate, tannin and saponin contents of the control feed (100% Commercial top starter mash feed), *Cirina forda* larva powder and that of 20% and 80% *Cirina forda* diets.

Table 4.21: Proximate composition of *Cirina forda*powder and rats feed used (g/100g)

Parameter	Sample A 100% Commercial Top-feed starter mash	Sample B Mixture of 20% *C. forda* powder + Top-feed starter mash	Sample C Mixture of 80% *C. forda* powder + Top-feed starter mash	Sample D *C. forda* powder
Moisture	10.59 ± 0.03^{a}	9.34 ± 0.03^{b}	9.12 ± 0.03^{c}	5.85 ± 0.04^{d}
Protein	22.28 ± 0.11^{a}	23.79 ± 0.10^{b}	24.28 ± 0.11^{c}	52.98 ± 0.20^{d}
Fat	8.70 ± 0.27^{a}	9.10 ± 0.02^{b}	10.87 ± 0.02^{c}	14.13 ± 0.02^{d}
Crude Fibre	4.11 ± 0.03^{a}	3.93 ± 0.03^{b}	3.72 ± 0.04^{c}	0.90 ± 0.00^{d}
Ash	1.78 ± 0.06^{a}	2.24 ± 0.02^{b}	2.65 ± 0.02^{c}	2.05 ± 0.03^{d}
Carbohydrate	52.55 ± 0.32^{a}	51.62 ± 0.06^{b}	49.36 ± 0.05^{c}	24.08 ± 0.14^{d}
Gross Energy (Kcal)	411.40 ± 0.20^{a}	432.67 ± 0.21^{b}	459.03 ± 0.15^{c}	448.97 ± 0.40^{d}
Dietary Fibre	17.09 ± 0.03^{a}	16.95 ± 0.03^{b}	15.21 ± 0.03^{c}	8.51 ± 0.03^{d}

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Reported values are means±standard deviationof three determinations (n=3).

Table 4.19: Mineral content of rat feed and *Cirina forda* powder (mg/100g)

PARAMETER	Sample A 100% Commercial Top-feed starter mash	Sample B Mixture of 20% *C. forda* powder + Top-feed starter mash	Sample C Mixture of 80% *C. forda* powder + Top-feed starter mash	Sample D *C. forda* powder
Sodium	210.33±1.53[a]	218.67± 4.04[b]	223.00 ± 2.00[c]	290.0 ± 2.00[d]
Potassium	606.00±2.00[a]	621.00±3.61[b]	625.33 ±3.06[c]	623.67 ± 3.06[d]
Calcium	958.333.51[a]	969.33 ±2.52[b]	991.00 ±4.00[c]	262. 00± 3.00[d]
Phosphorus	367.67±1.53[a]	377.00 ± 2.00[b]	391.66±2.52[c]	146.67 ± 1.53[d]
Magnesium	218.00±3.00[a]	230.67 ± 2.52[b]	231.00 ± 3.00[b]	278.67± 3.51[c]
Iron	2.07 ±0.02[a]	2.20 ±0.03[b]	2.34 ±0.02[c]	8.94 ± 0.02[d]
Copper	0.43±0.02[a]	0.52 ±0.03[b]	0.56±0.03[c]	0.33 ± 0.02[d]
Zinc	1.94 ± 0.02[a]	2.11 ± 0.03[b]	1.93 ± 0.02[a]	1.39 ± 0.03[c]
Selenium (µg/)	0.004±0.00[a]	0.004 ± 0.00[a]	0.005 ±0.00[b]	0.01±0.00[c]

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Reported values are means±standard deviation of three determinations (n=3).

Table 4.20: Vitamin composition of rat feed and *Cirina forda* powder (mg/100g)

Parameter	Sample A 100% Commercial Top-feed starter mash	Sample B Mixture of 20% *C. forda* powder + Top-feed starter mash	Sample C Mixture of 80% *C. forda* powder + Top-feed starter mash	Sample D *C. forda* powder
Vitamin A (µg/)	312.71 ± 0.02[a]	318.87±0.02[b]	327.81 ± 0.03[c]	0.33 ± 0.02[d]
Vitamin B1	0.57 ± 0.02[a]	0.49±0.03[b]	0.43 ± 0.02[c]	0.19 ± 0.02[d]
Vitamin B2	0.14±0.00[a]	0.13±0.00[b]	0.12 ±0.00[c]	0.37 ± 0.01[d]
Vitamin B3	8.87 ± 0.03[a]	8.61±0.03[b]	7.29±0.03[c]	0.62 ± 0.03[d]
Vitamin B6	0.17 ±0.00[a]	0.16 ± 0.00[b]	0.15 ±0.00[c]	0.46±0.02[d]
Vitamin B12	7.86 ± 0.02[a]	8.01±0.04[b]	8.77± 0.02[c]	3.53± 0.02[d]
Vitamin C	2.07 ± 0.03[a]	1.95 ± 0.03[b]	1.91±0.02[c]	19.24±0.03[d]
Vitamin E (µg/)	29.77±0.02[a]	31.72±0.03[b]	33.57±0.02[c]	1.89 ± 0.03[d]
Vitamin K (µg/)	6.82 ±0.05[a]	6.92 ±0.04[b]	7.07 ± 0.03[c]	0.33 ±0.02[d]

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Reported values are means±standard deviationof three determinations (n=3).

Table 4.21: Antinutrient content in the feeds and *Cirina forda* powder(mg/100g)

Parameter	Sample A 100% Commercial Top-feed starter mash	Sample B Mixture of 20% *C. forda* powder + Top-feed starter mash	Sample C Mixture of 80% *C. forda* powder + Top-feed starter mash	Sample D *C. forda* powder
Phytate	0.01 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.01 ± 0.00^{a}
Oxalate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Tannin	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
Saponin	0.17 ± 0.00^{a}	0.16 ± 0.00^{b}	0.16 ± 0.00^{b}	0.00 ± 0.00^{a}
Trypsin Inhibitor	1.52 ± 0.04^{a}	1.59 ± 0.03^{b}	1.72 ± 0.03^{c}	0.56 ± 0.03^{d}

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Reported values are means$\pm$standard deviation of three determinations (n=3).

4.22 Phytochemical Screening of *Cirina forda*liquid extract

Phytochemical screening of *Cirina forda*liquid extract (Table 4.25) showed that alkaloids, tannin, saponin and phenol in addition to phylobatanin, flavonoids, anthraquinones, steroids, terpenes, and cardiac glycosides were existing in the *Cirina forda*liquid extract in appreciable amounts, but charcones and cardenolides were completely absent.

Table 4.22: Phytochemical Screening of *Cirina forda*liquid extract

Phytochemicals	Observation	Phytoquantitation
Alkaloids	+++	0.52 ± 0.00
Tannin	+++	0.03 ± 0.01
Phlobatannin	++	0.01 ± 0.00
Saponin	+++	0.42 ± 0.00
Flavonoids	++	0.01 ± 0.00
Anthraquinones	++	0.00 ± 0.00
Steroids	++	0.00 ± 0.00
Terpenes	++	0.00 ± 0.00
Cardenolides	-	
Phenolics	+++	0.17 ± 0.01
Chalcones	-	
Cardiac Glycosides	++	0.23 ± 0.00

Key:

(+++) = present in an appreciable amount;

(++) = present in a moderate amount;

(+) = present in a minute amount;

(-) = Completely absent.

4.23 Histopathological study of rats organs in the repeated feeds intake

The histopathology of rats' brains on tested feeds and control is shown in Plate 4.1. The slides of brains of rats on the 20% *Cirina forda*powder inclusion level (Plate 4.1(a)) showed no visible lesion in the first, second and third slides while a mild diffuse spongiosis of the brain parenchyma, a moderate to severe congestion/haemorrhage of the meninges, and mild gliosis (submeningeal) was observed in the fourth slide.In the slides of rats on 80% *Cirina forda*powder inclusion feed (Plate 4.1(b)), there was a mild diffuse spongiosis of the brain parenchyma in the first slide, while in the fourth slide, no visible lesionswere seen, but there was mild congestion of the meninges. However, no visible lesions wereseen in the brains of rats in the control group (Plate 4.1(c)).

Plate 4.2 showed the histopathological effects of the feeds on the rats' kidneys. In Plate 4.2 (a), the kidneys of the rats fed with 20% *Cirina forda*powder inclusion level showed no visible lesion in the first and second slides. There were protein casts in the tubular lumen of the convoluted tubules in the third slide, while there was a mild to moderate interstitial congestion and haemorrhage in the fourth slide. For the 80% *Cirina forda*inclusion level (Plate 4.2(b)), no visible lesion was observed in the first slide while there was severe interstitial congestion with mild peritubular cellular infiltration in the fourth slide. No visible lesion was observed in the kidneys of the control group (Plate 4.2(c)).

Plate 4.3 showed the pathological effects of the feeds on rats' liver. For 20% *Cirina forda*powder inclusion feed (Plate 4.3(a)),no visible lesions were seen in the first slide, mild cellular infiltration of the portal area wasobserved in the second slide, a mild periportal fibrous proliferation was seen in the third slide,while the sinusoids appeared congested and infiltrated in the fourth slide.In the 80% *Cirina forda* inclusion feed group (Plate 4.3(b)), there was moderate portal congestion with very mild periportal cellular infiltration in the first slide, while there was very severe portal congestion in the fourth slide.No visible lesion was observed in the control slides (Plate 4.3(c)).

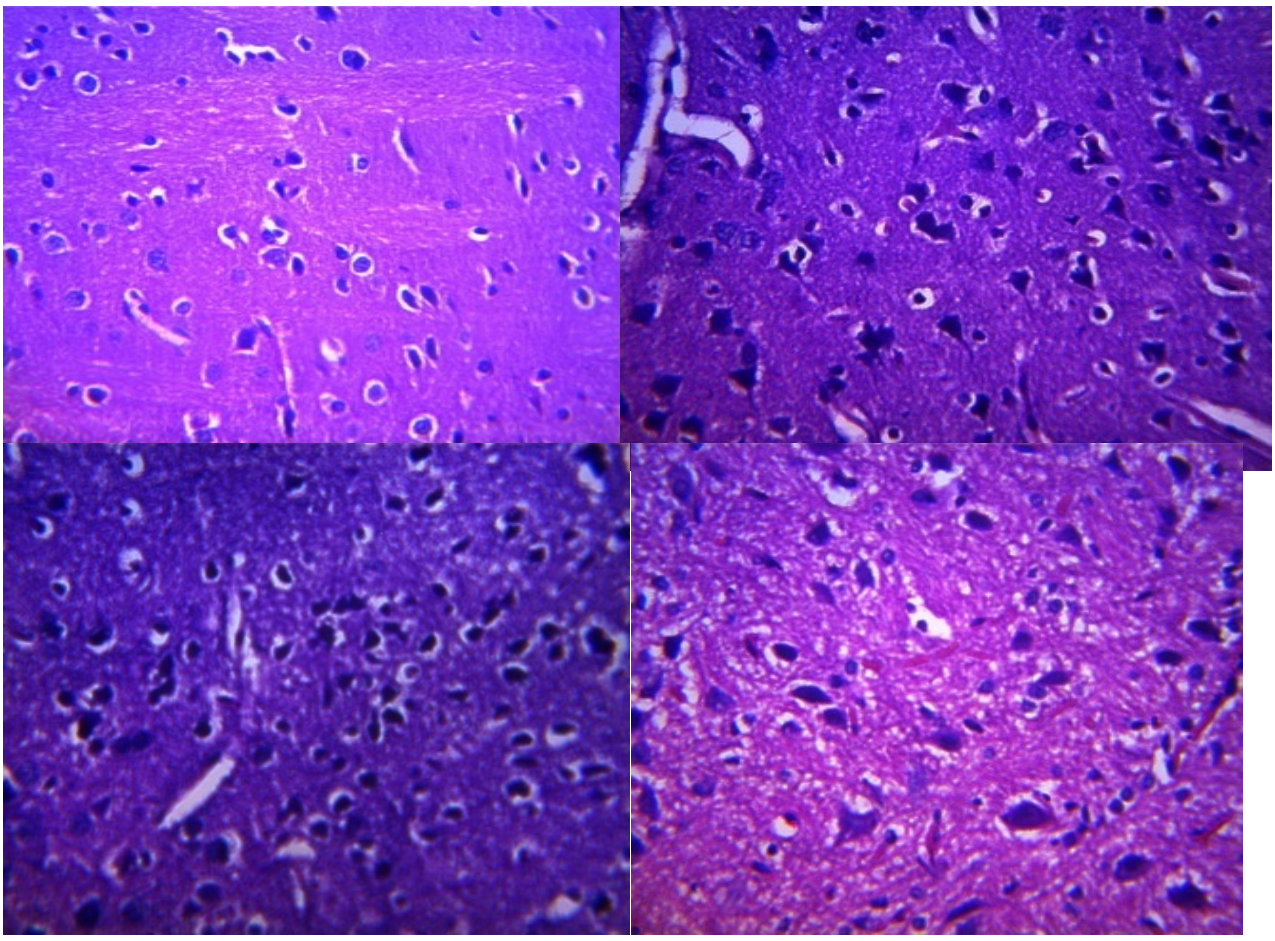


Plate 4.1 (a): Brains of rats on 20% *Cirina forda*inclusion level

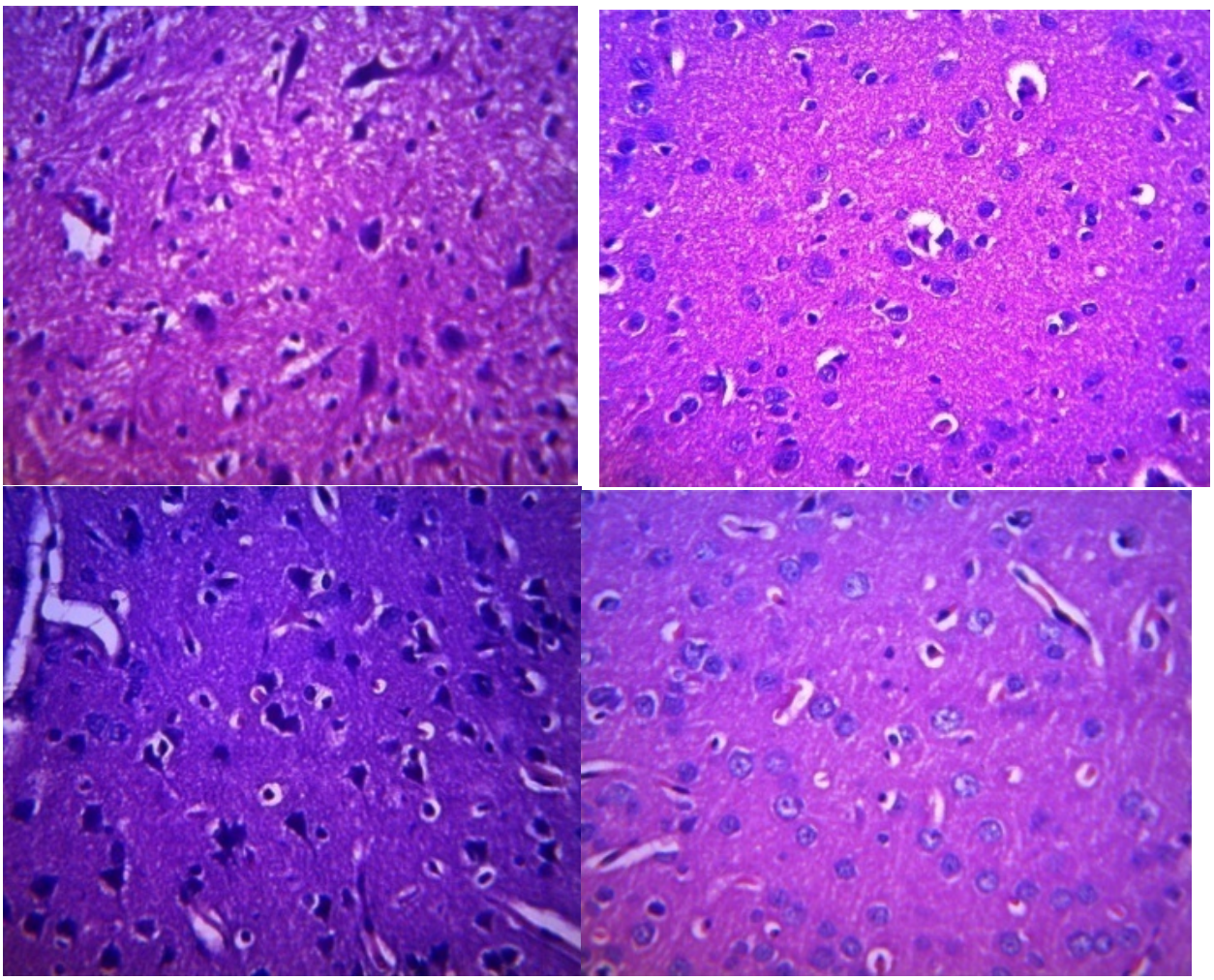


Plate 4.1 (b): Brains of rats on 80% *Cirina forda*inclusion level

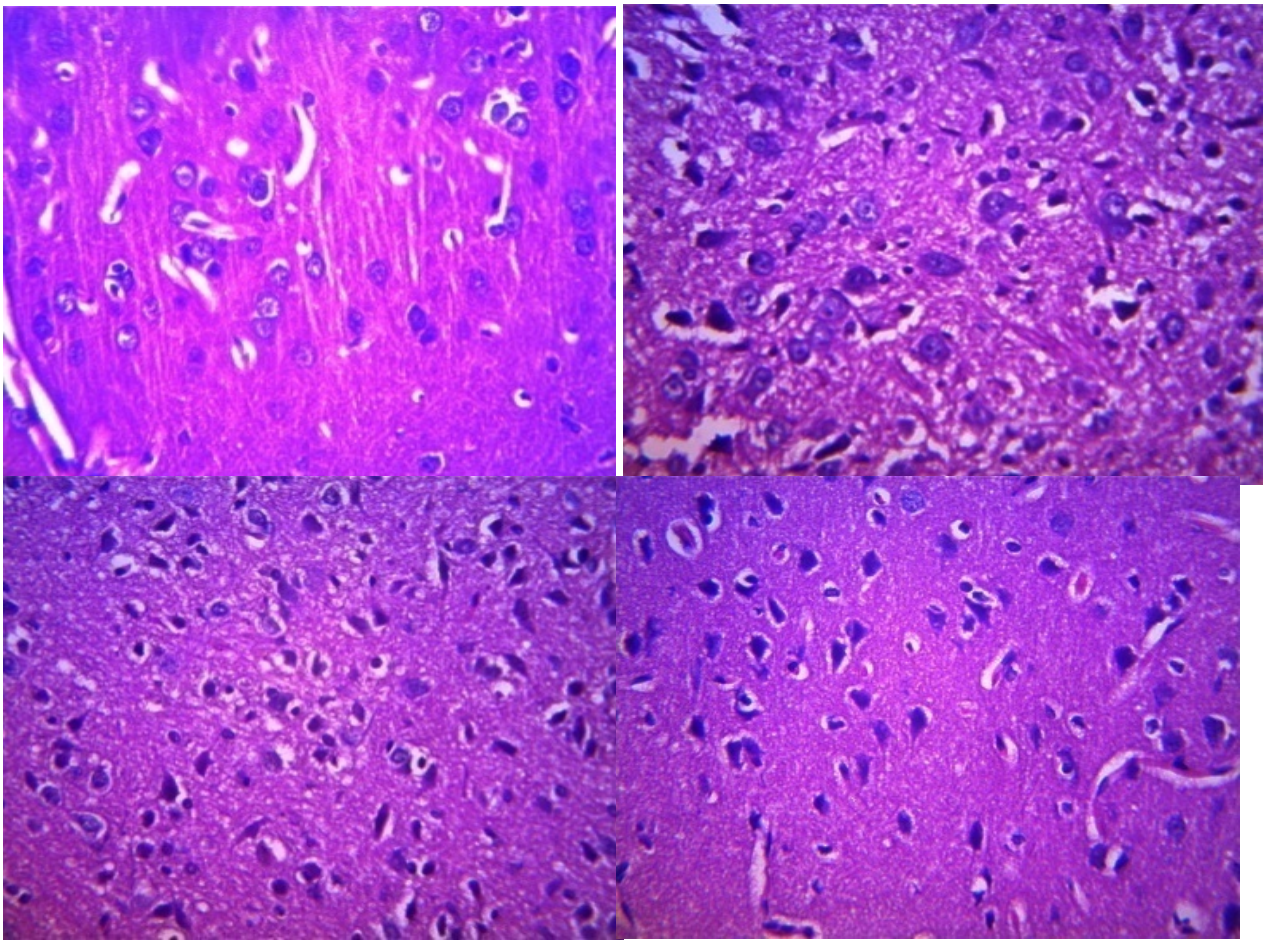


Plate 4.1(c): Brains of rats on 100% commercial rat feed (Control)

Plate 4.1: Histopathology slides of the brain of experimental animals

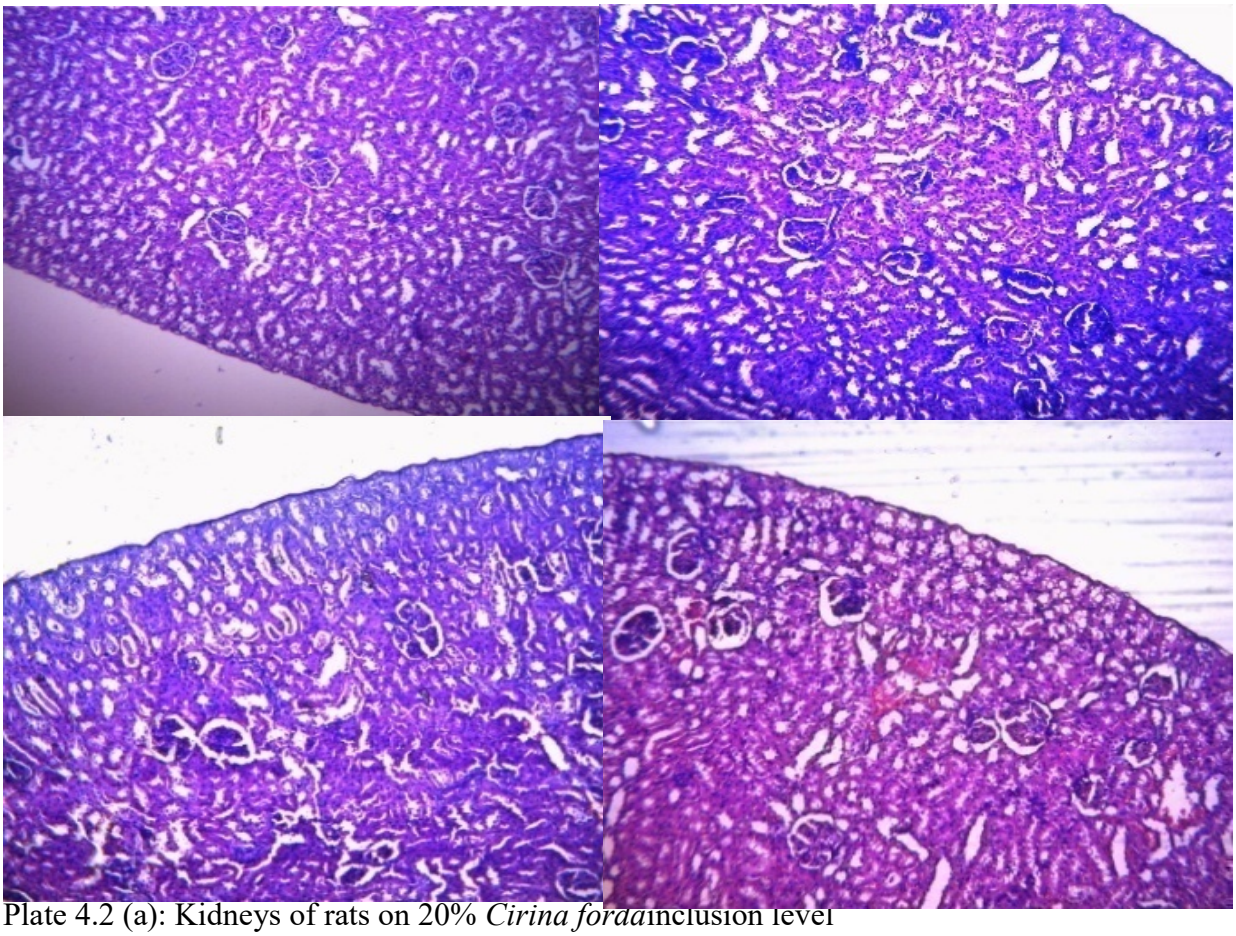


Plate 4.2 (a): Kidneys of rats on 20% *Cirina forda*inclusion level

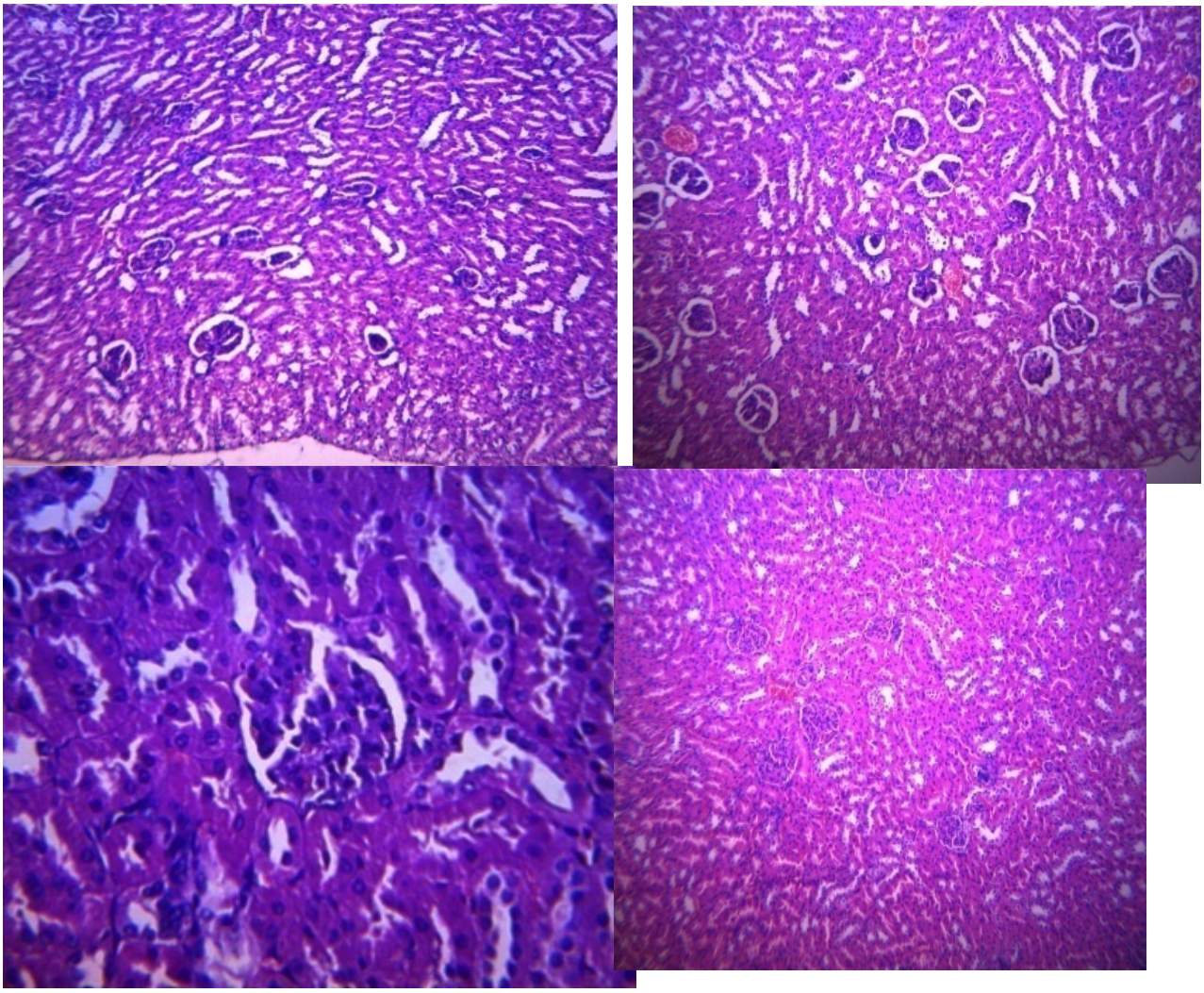


Plate 4.2 (b): Kidneys of rats on 80% *Cirina forda*inclusion level

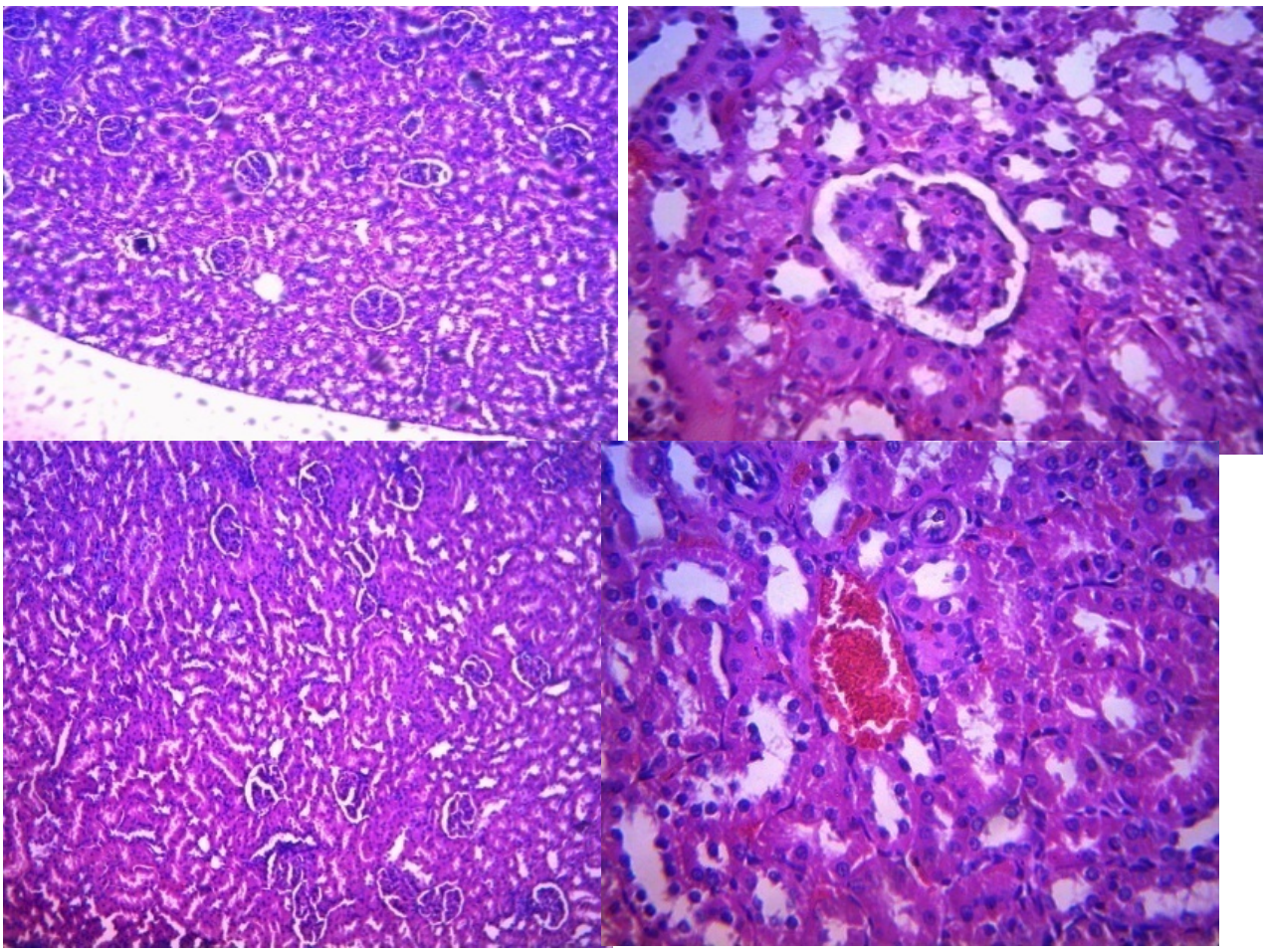


Plate 4.2 (c): Kidneys of rats on 100% commercial rat feed (Control)

Plate 4.2:Histopathology slides of the Kidneys of experimental animals

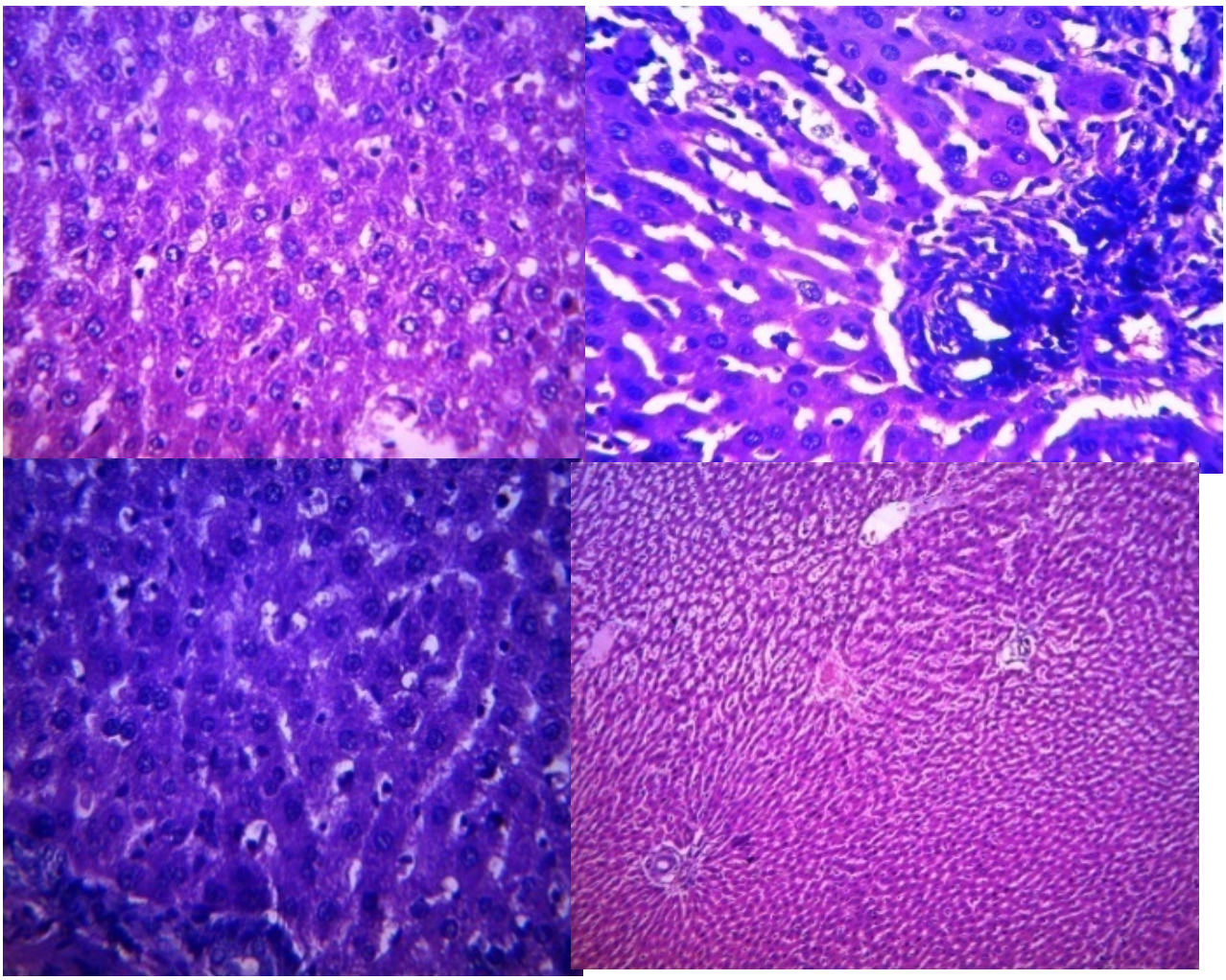


Plate 4.3 (a): Livers of rats on 20% *Cirina forda*inclusion level

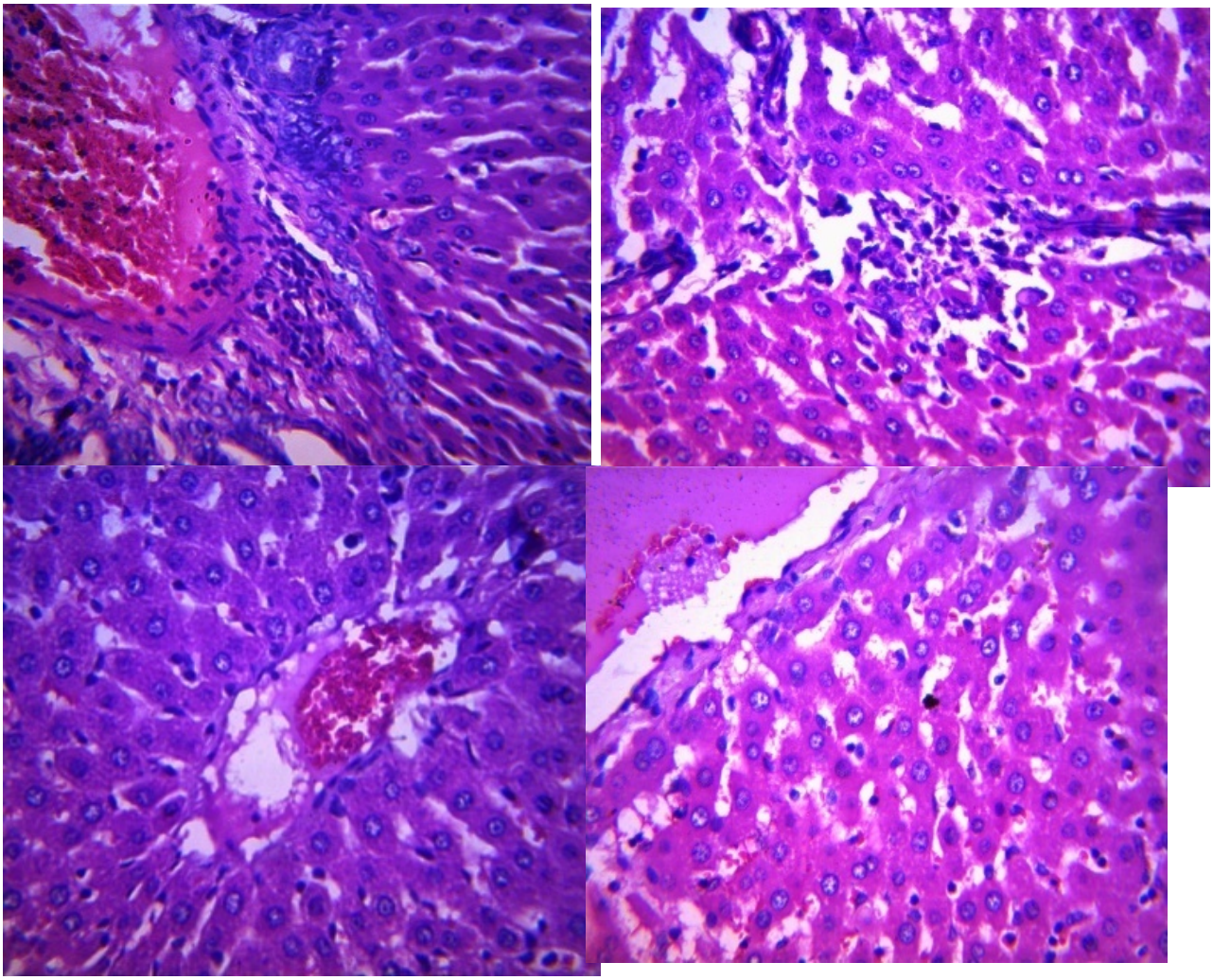


Plate 4.3 (b): Livers of rats on80% *Cirina forda*inclusion level

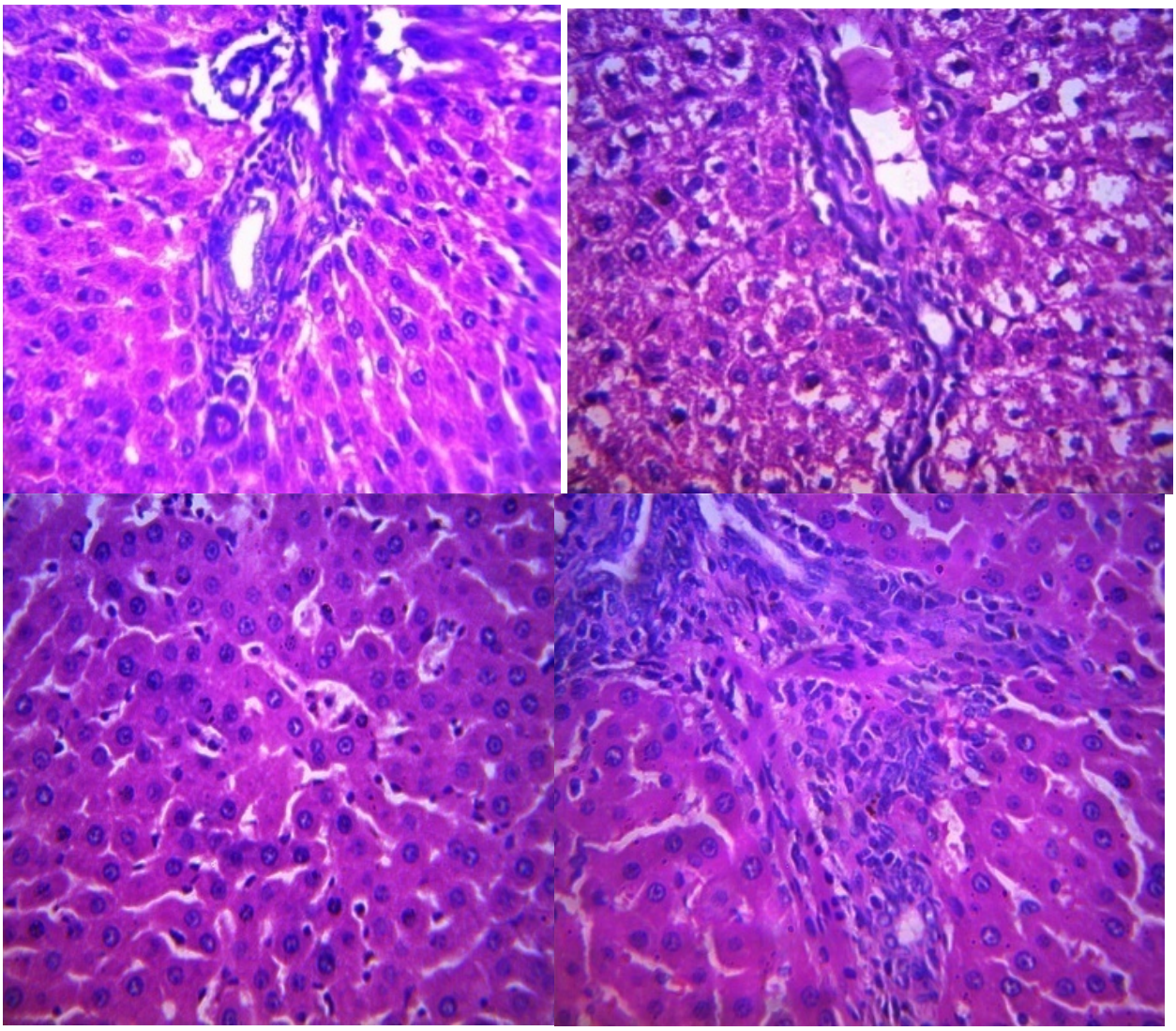


Plate 4.3 (c): Liver of rats on 100% commercial rat feed (Control)

Plate 4.3: Histopathology slides of the Liver of experimental animals

Specific Objective 4

To evaluate the nutrient bioavailability of *Cirina forda* larvae using experimental rats

4.24 Mean feed intake and weight gain of rats

All the group diets or feeds except for the protein-free (Basal) group contained 15% crude protein (N x 6.25). Rats on the control diet consumed more feed than the rats on the experimental and basal diets. Rats on the basal diet consumed the least quantity of feed when compared with the other groups of Rats. The basal diet group lost approximately 10g mean weight after twenty-eight days duration of the experiment.The mean feed intake of the rats is shown in figure 4.2. The Control group had the highest feed intake (6.2g) while the basal diet group had the least feed intake (4.52g). Also, the mean weight gain of the rats (fig. 4.4) showed a significant weight gain in both experimental and control groups with the experimental G group having the highest weight gain (26.66g), ($p<0.05$), while rats on basal diet group recorded significant weight loss (-9.5g), ($p<0.05$).

4.25 Serum Nutrient content of Rats

Generally, Table 4.26 showed that the serum nutrient content of rats in this study differed significantly but no significant difference was observed among the experimental (S and G) diet groups ($p>0.05$) for serum protein, calcium and iron that showed ($P<0.05$)

4.26 Consumption index (CI), Growth rate (GR), and Efficiency of conversion (ECI) of Ingested feed.

Table 4.27 showed that the basal diet group had the highest CI while the experimental diets which can *Cirina forda*samples from Saki and Ghana had the least values. However, High GR and ECI were observed in the experimental diets compared to the basal diet. Also, the experimental, G diet had a net protein ratio (NPR) of 18.16 while the experimental S diet hadan NPR of 13.16.

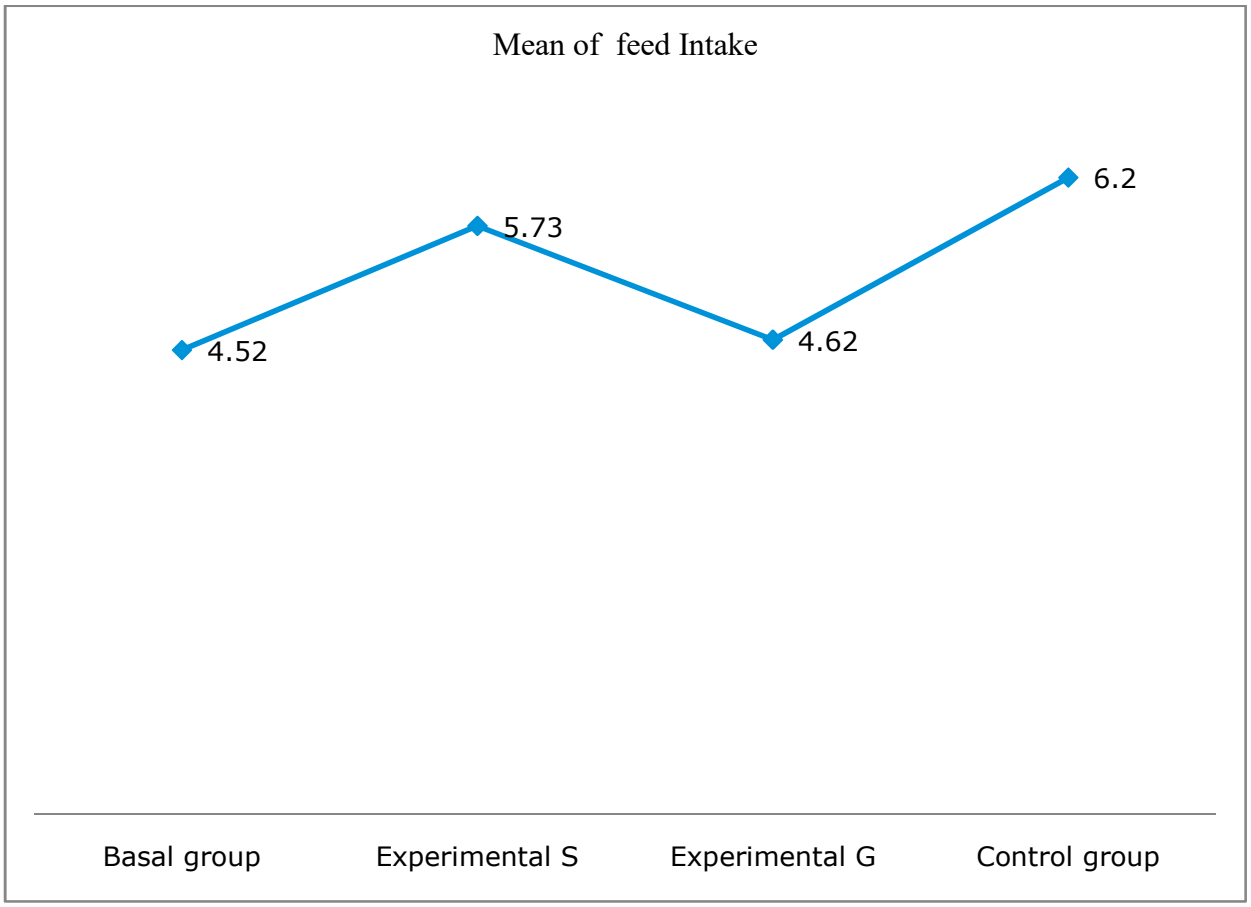


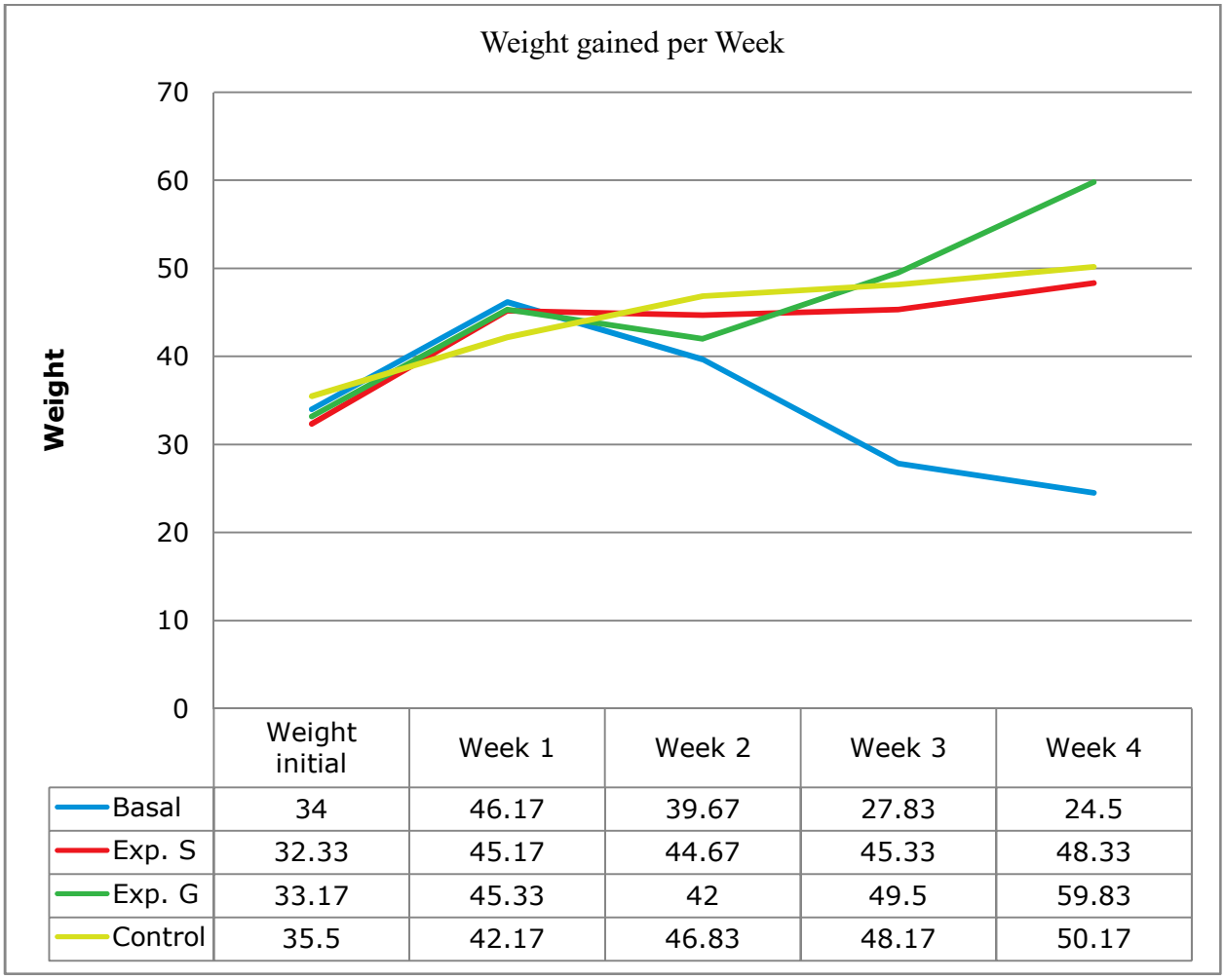
Fig. 4.2: Mean feed intake of rats(g)

Experimental S- feed formulated with *Cirina forda* sample from Saki

Experimental G – feed formulated with *Cirina forda* sample from Ghana

Control Group – Feed formulated with 100% casein diet

Basal Group – Feed with no protein diet.



	Weight initial	Week 1	Week 2	Week 3	Week 4
Basal	34	46.17	39.67	27.83	24.5
Exp. S	32.33	45.17	44.67	45.33	48.33
Exp. G	33.17	45.33	42	49.5	59.83
Control	35.5	42.17	46.83	48.17	50.17

Fig. 4.3: Weight of rats per week(g)

Experimental S- feed formulated with *Cirina forda* sample from Saki

Experimental G – feed formulated with *Cirina forda* sample from Ghana

Control Group – Feed formulated with 100% casein diet

Basal Group – Feed with no protein diet

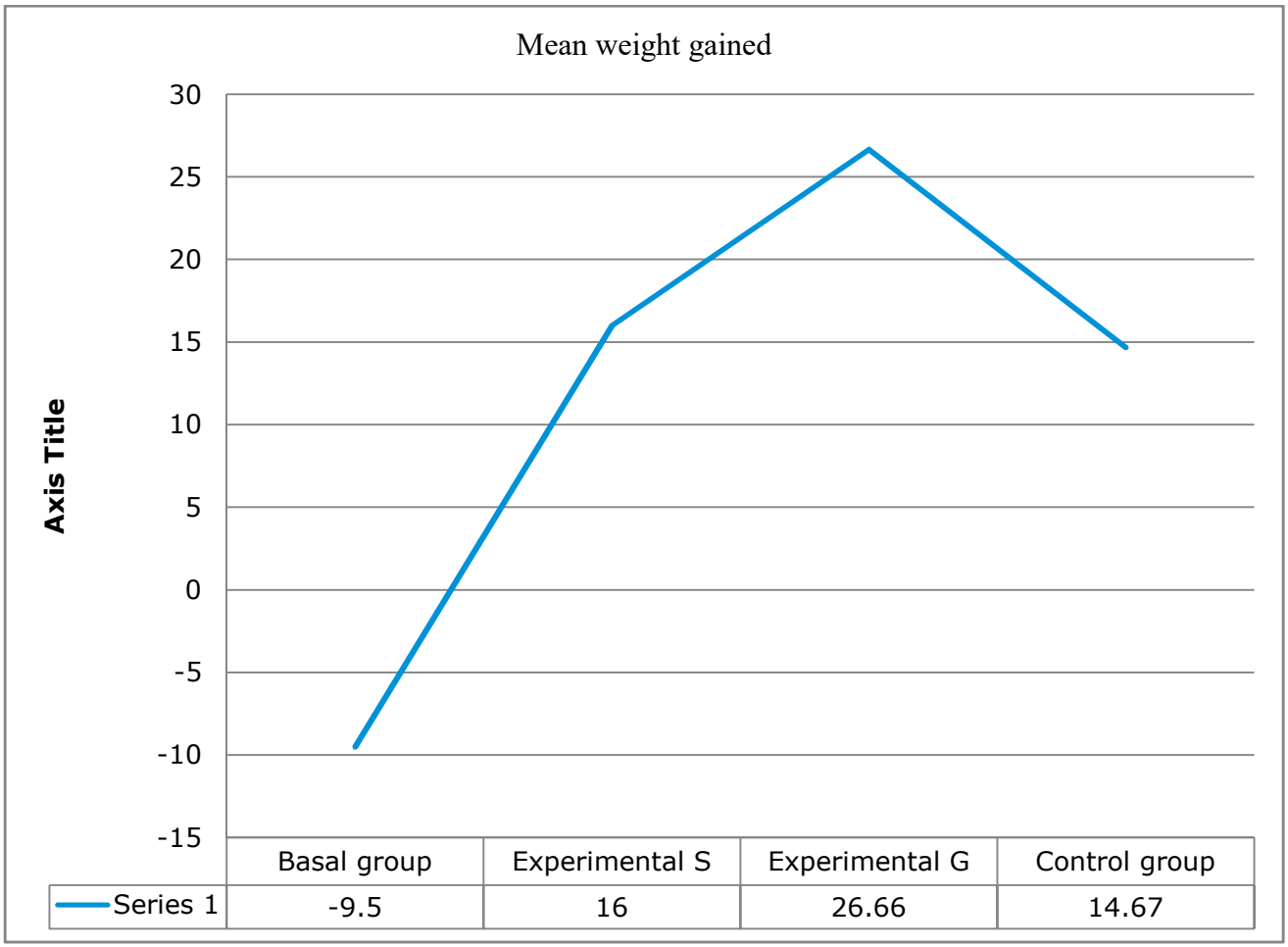


Fig 4.4: Mean weight gain of rats(g)

Experimental S- feed formulated with *Cirina forda* sample from Saki

Experimental G – feed formulated with *Cirina forda* sample from Ghana

Control Group – Feed formulated with 100% casein diet

Basal Group – Feed with no protein diet

Table 4.23: Serum Nutrient content of Rats (g or mg/dL)

Parameters	Basal diet	Experimental Saki diet, S	ExperimentalGhana diet,G	Control, 100% casein diet
Protein	4.06 ± 0.14[a]	4.83 ± 0.41[b]	5.10 ± 0.14[b]	5.54 ± 0.21[c]
Calcium	24.28 ± 0.70[a]	26.82 ± 0.68[b]	26.78± 0.70[b]	28.60 ± 0.57[c]
Zinc	3.10 ± 0.49[a]	4.50 ± 0.51[b]	5.64 ± 0.44[c]	6.22 ± 0.24[c]
Iron	22.42 ± 1.37[a]	25.32 ± 0.51[b]	26.04 ± 0.55[b]	27.72 ± 0.85[c]
Vitamin A	31.14 ± 0.48[a]	36.10 ± 0.45[b]	40.16 ± 0.99[c]	47.30 ± 0.72[d]

Values with similar alphabets along the same row are insignificantly different ($p>0.05$). Reported values are means±standard deviation of three determinations ($n=3$).

Table 4.24: Consumption index, Growth rate, and Efficiency of conversion of Ingested feed

Parameters	Basal diet	Experimental Saki diet, S	Experimental Ghana diet, G	Control, 100% casein diet
Conversion index, CI	0.0062	0.0059	0.0045	0.0060
Growth rate, GR	0.00074	0.0141	0.0155	0.0115
Efficiency of conversion of Ingested feed, ECI	11.95	236.30	346.10	182.90
Net protein ratio, NPR	-	13.16	18.16	-

4.27 Histopathological study of rats' organs

Plate 4.4 showed the histopathology slides of the Heart of experimental Animals. The basal diet group showed that cardiomyocytes appearance was normal and no visible lesions were seen in the first slide. In the second slide, there were a few foci of mild clear cytoplasmic vacuolation of cardiomyocytes. In the third slide, there were a few foci of degeneration and loss of striations of cardiomyocytes, while in the fourth slide, cardiomyocytes appeared normal, however, there is marked congestion of coronary blood vessels. In the Shaki *Cirina forda*diet group, cardiomyocytes appear normal, however, there were extensive epicardialhaemorrhages in the first slide, while in the second slide, there are multiple foci of clear vacuolation of cardiomyocytes. In the Ghana *Cirina forda*diet group, no evident lesion was observed in the slide. In the Control diet group, cardiomyocytes appeared normal in the first two slides and no visible lesion was seen in all the slides.

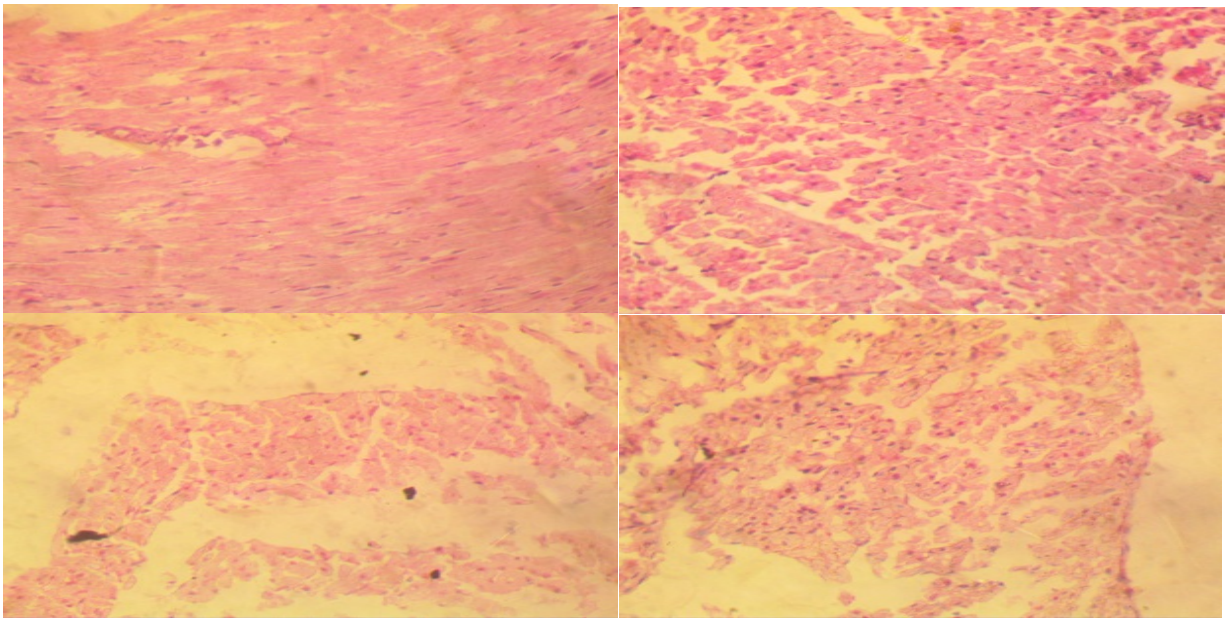
Plate 4.5 showed the histopathology slides of the Intestine of experimental Animals. In the basal diet group,some numerous closely-packed tall villi and crypts appeared normal in the first and fourth slides. In the second slide, villi and crypts appear normal. In the third slide, the villi are few and stunted and there is mild necrosis of the crypts.In the Shaki *Cirina forda*diet group, therewere few foci of loss of villi and moderately reduced cryptal depth in the first slide, however, intact villi are tall. In the second slide, there are moderate numbers of closely-packed short villi and crypts that appeared normal. In the third slide, there are numerous villi although they appear slightly reduced in height and there is mild necrosis of crypts. There is moderate congestion of submucosal blood vessels. There is a helminth in the lumen of the intestine while in the fourth slide, there is severe necrosis and loss of the intestinal villi and crypts.In the Ghana*Cirina forda*diet group, there are numerous closely-packed villi and increased cryptal depth and no visible lesion seen in the first slide. In the second slide, there are moderate numbers of short but intact villi and the cryptal depth is reduced although the crypts appear normal. In the third slide, there was numerous very tall closely-packed villi and no visible lesion seen, while in the fourth slide, there are numerous closely-packed tall villi, crypts appear normal and no visible lesion seen.In the Control diet group, there are numerous closely-packed tall villi, crypts appear normal and no visible lesion seen in the first slide. In the second slide, there were numerous villi although they appear slightly reduced in height, the cryptal depth is markedly reduced and there

was a helminth in the lumen of the intestine**.** In the third slide, there are numerous villi although they appear slightly reduced in height, the cryptal depth was markedly reduced and there was a helminth in the lumen of the intestine. In the fourth slide, some numerous closely-packed tall villi and crypts appeared normal

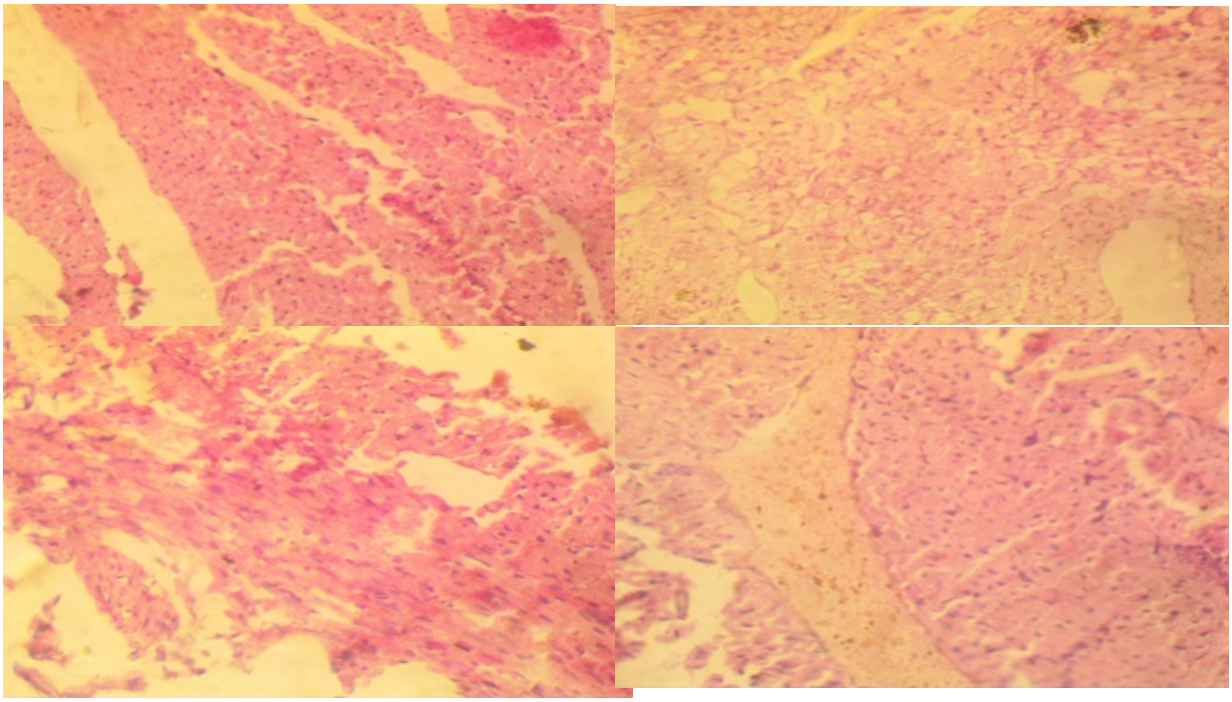
Plate4.6 showed the histopathology of the Kidney of experimental Animals. In the Basal diet group, Glomeruli and tubules showed no visible lesions in the first and fourth slides. In the second slide, there were multiple foci of marked sloughing off of tubular epithelial cells with the remnant basement membrane intact, Glomeruli and renal interstitium appeared normal. In the third slide, there are a few foci of sloughing off of tubular epithelial cells and blocking of renal interstitial blood vessels.Also, Glomeruli and tubules of the Shaki*Cirina forda*diet group showed no visible lesion in the first slide. In the second slide, there are a few foci of loss of tubular epithelial cells while in the third slide, there are a few foci of marked sloughing off of tubular epithelial cells with the remnant basement membrane intact. In the Ghana*Cirina forda*diet group, there are multiple foci of marked sloughing off of tubular epithelial cells with the remnant basement membrane intact, Glomeruli and renal interstitium appear normal in the first slide**.** In the second slide, there are a few foci of mild to moderate flattening of tubular epithelium.In the Control diet group,Glomeruli and tubules show no visible lesion in all the slides.

Plate 4.7 revealed the histopathology of the Liver of experimental Animals. In the Basal diet group,there wasa widespread moderate vacuolar change of hepatocytes in the first slide. In the second and fourth slides, hepatic plates were closely packed and no visible lesions were seen. In the third slide, there are multiple foci of moderate thinning of hepatic plates thus giving the sinusoids an expanded appearance and there wasa widespread moderate vacuolar change of hepatocytes giving the hepatocytes a foamy appearance.In the Shaki*Cirina forda*diet group, hepatic plates were closely-packed and there wasa widespread mild vacuolar change of hepatocytes giving the hepatocytes a foamy appearance in the first slide. In the second slide, hepatic plates were closely-packed and there were limited foci of insignificant vacuolar alteration of hepatocytes. In the third slide, hepatic plates were tight and there wasa widespread severe vascular change of hepatocytes giving the hepatocytes a foamy appearance, while in the fourth slide, hepatic plates were closely-packed and there wasa widespread mild vacuolar change of hepatocytes giving the hepatocytes a foamy appearance. In the Ghana*Cirina forda*diet group,

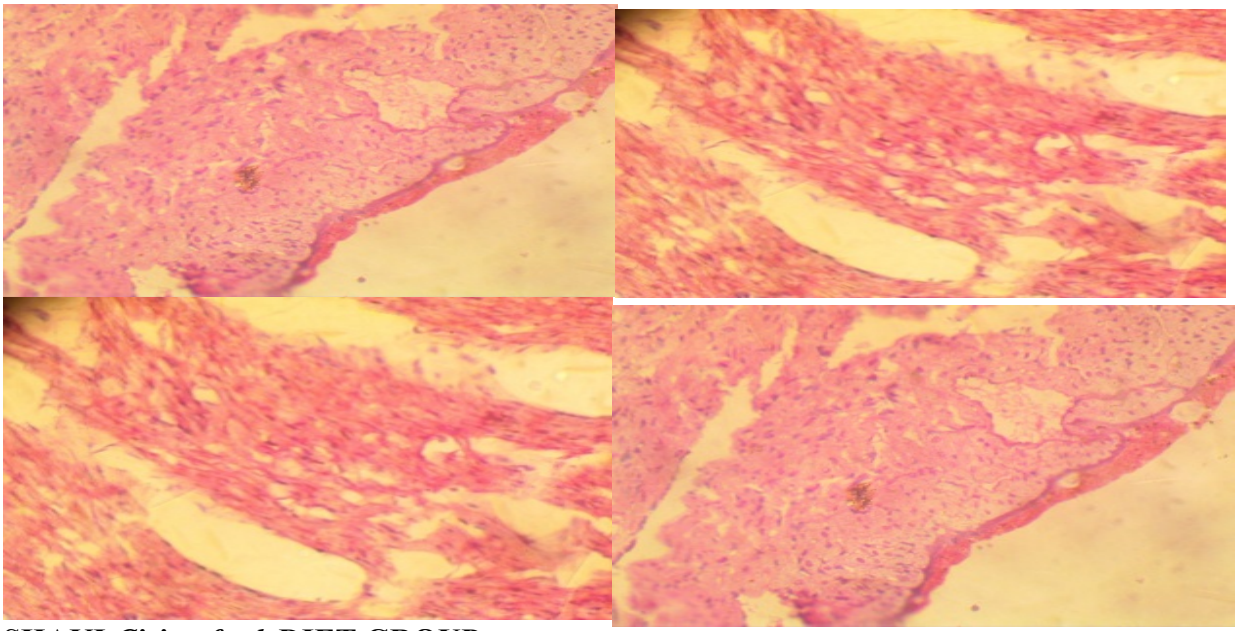
hepatic plates were closely-packed and there wasa widespread mild vacuolar change of hepatocytes giving the hepatocytes a foamy appearance in the first slide. In the second slide, hepatic plates were closely-packed and there are multiple foci of marked vacuolar change of hepatocytes. In the third slide, there wasa widespread severe vacuolar change of hepatocytes with the nuclei of the hepatocytes being pushed to the periphery, while in the fourth slide, hepatic plates are closely-packed and there is a widespread moderate vacuolar change of hepatocytes giving the hepatocytes a foamy appearance. In the Control diet group,hepatic plates were closely-packed and no visible lesion seen in the second slide. In the third slide, hepatic plates were closely-packed and there were limited foci of insignificant vacuolar alteration of hepatocytes, while in the fourth slide, hepatic plates are closely packed and there was moderate Kupffer cell hyperplasiam



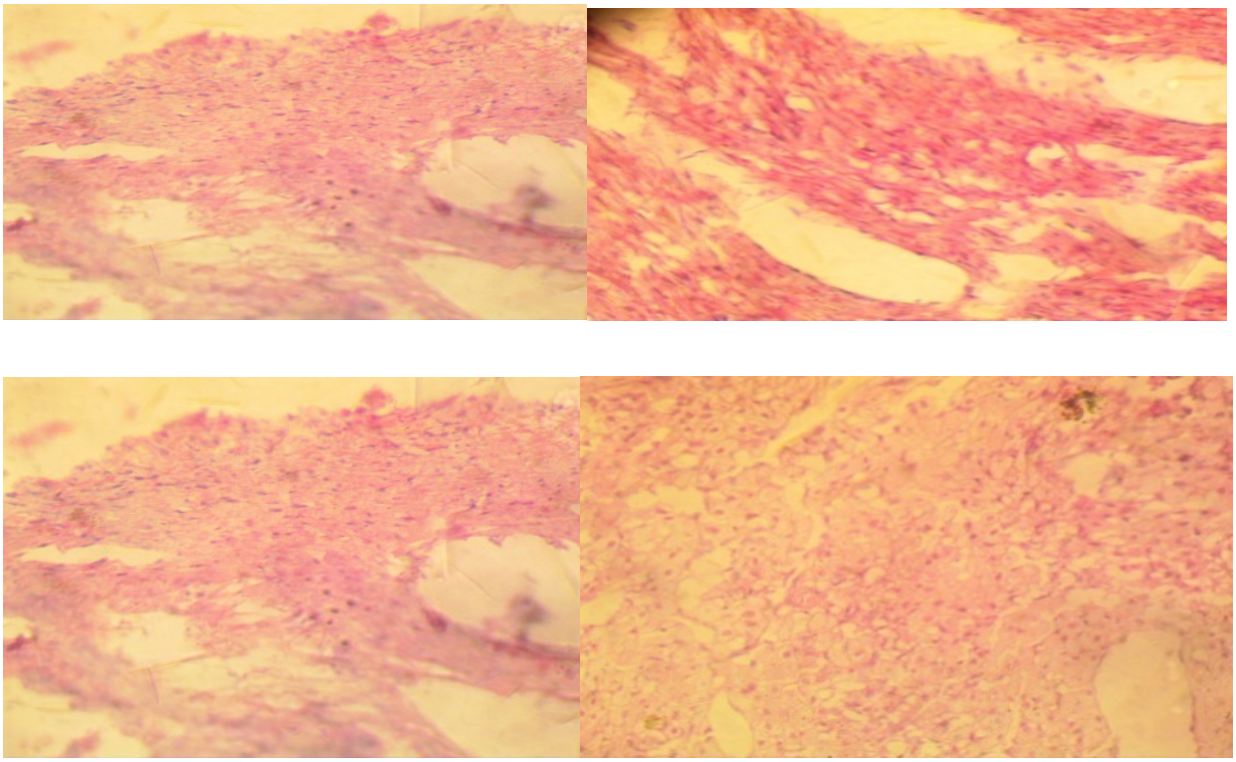
CONTROL DIET GROUP



BASAL DIET GROUP

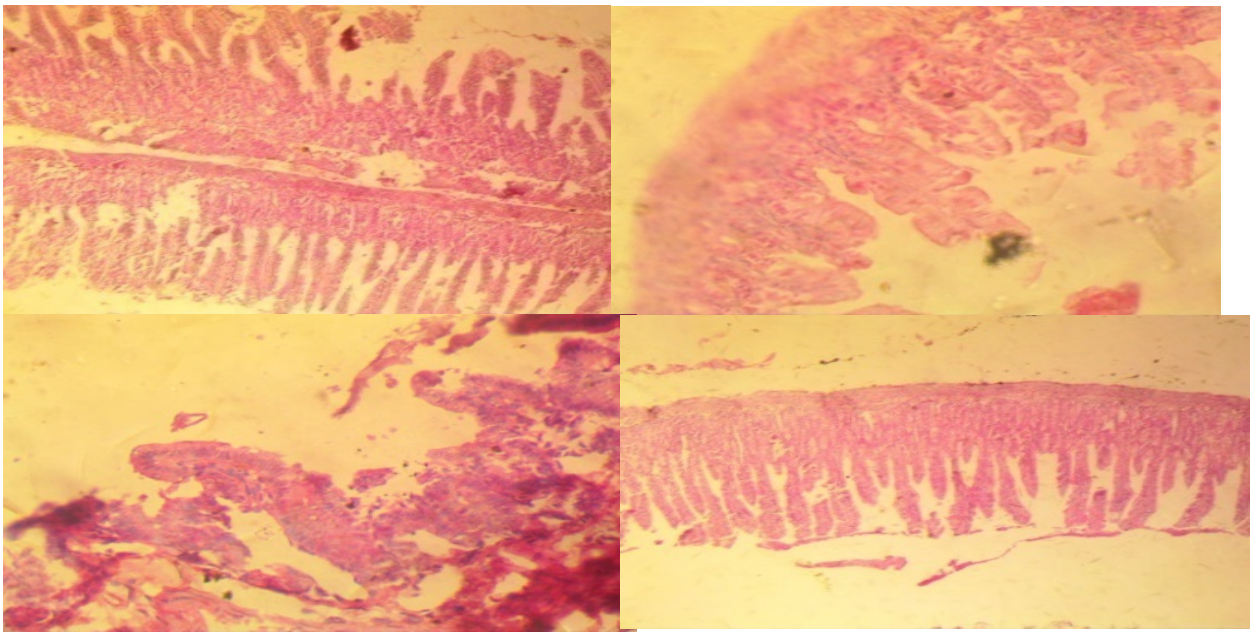


SHAKI *Cirina forda*DIET GROUP

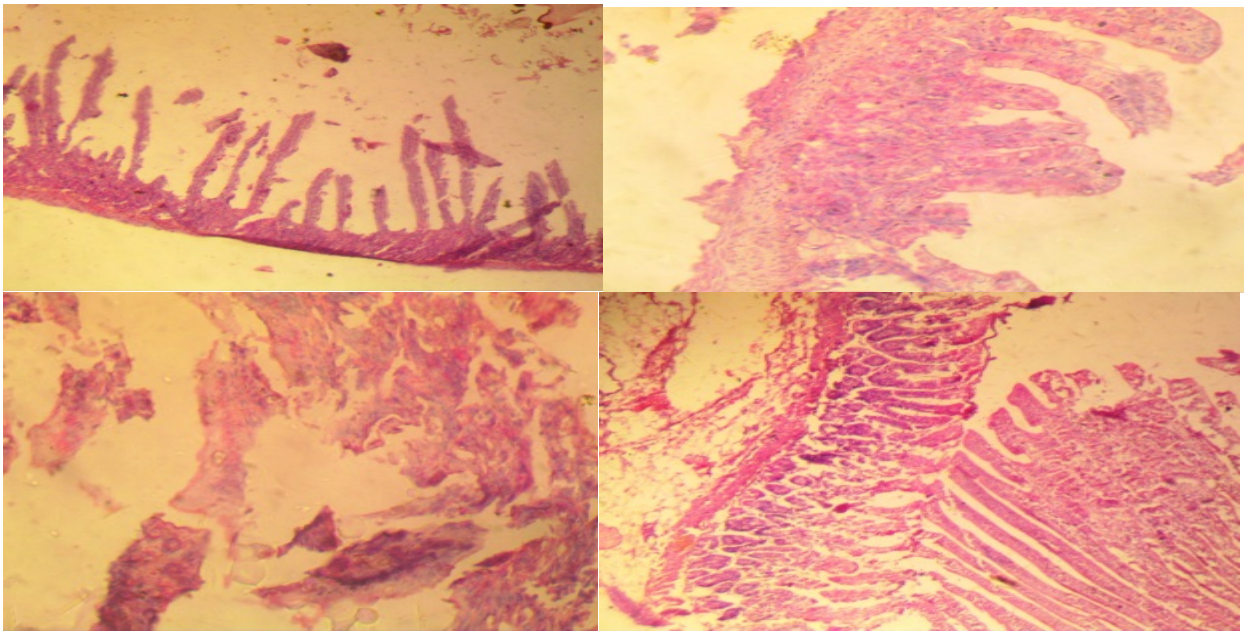


GHANA *Cirina forda*DIET GROUP

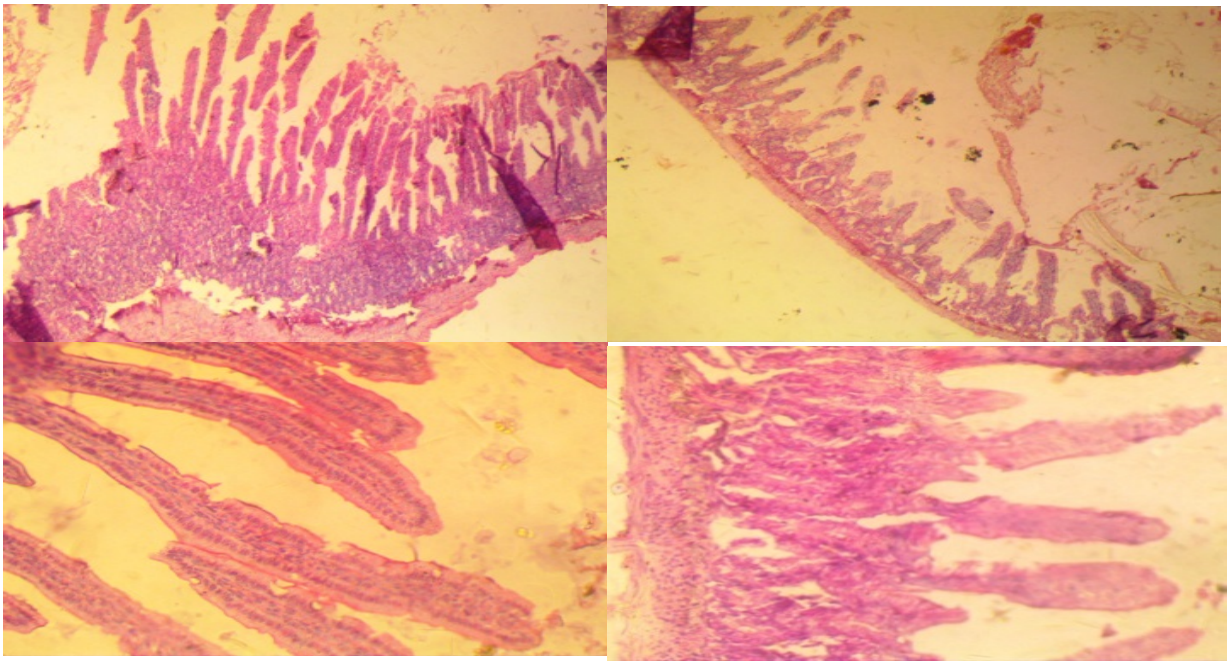
Plate 4.4: Histopathology slides of the rats' hearts



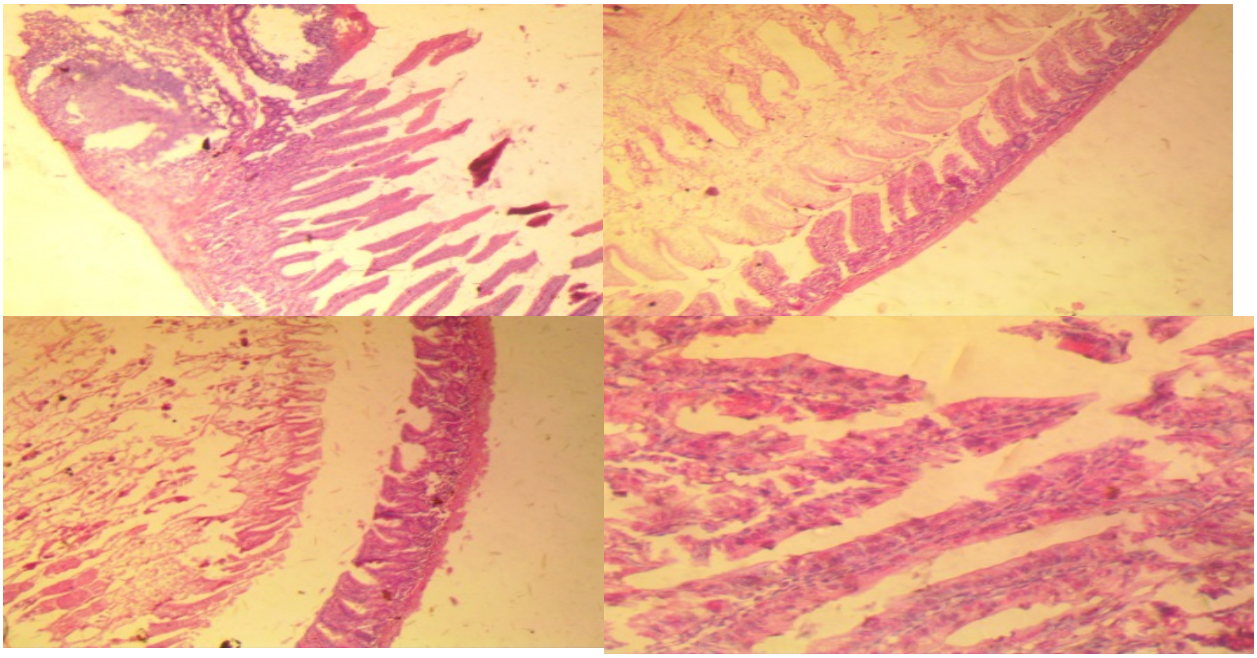
BASAL DIET GROUP



SHAKI *Cirina forda*DIET GROUP

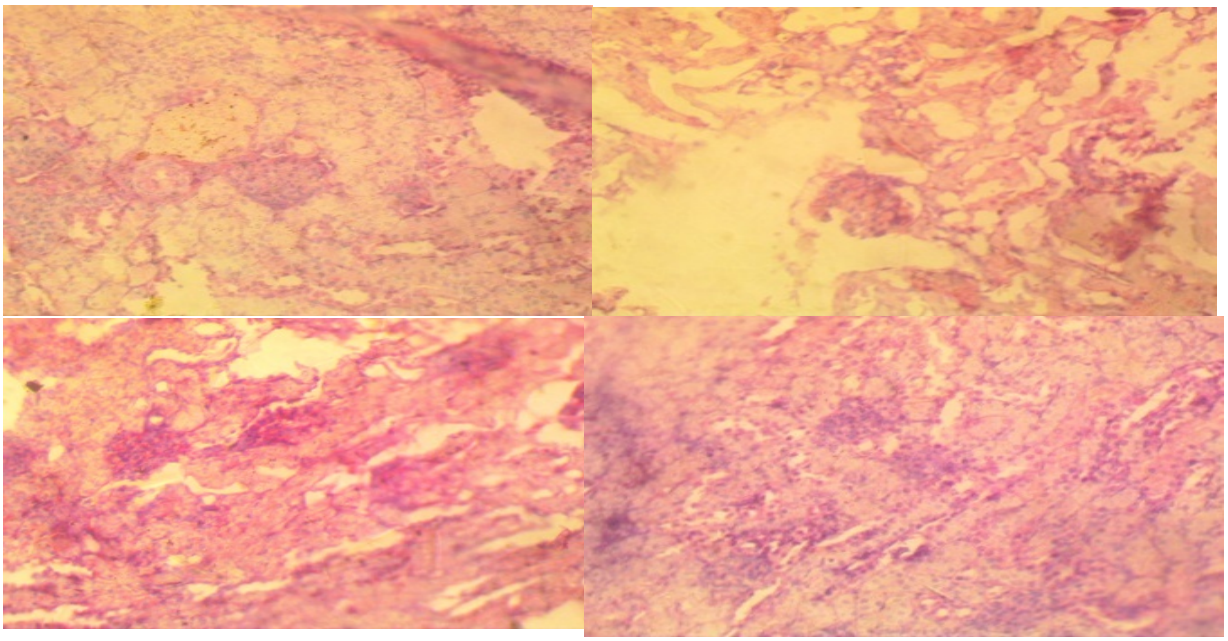


GHANA *Cirina forda*DIET GROUP

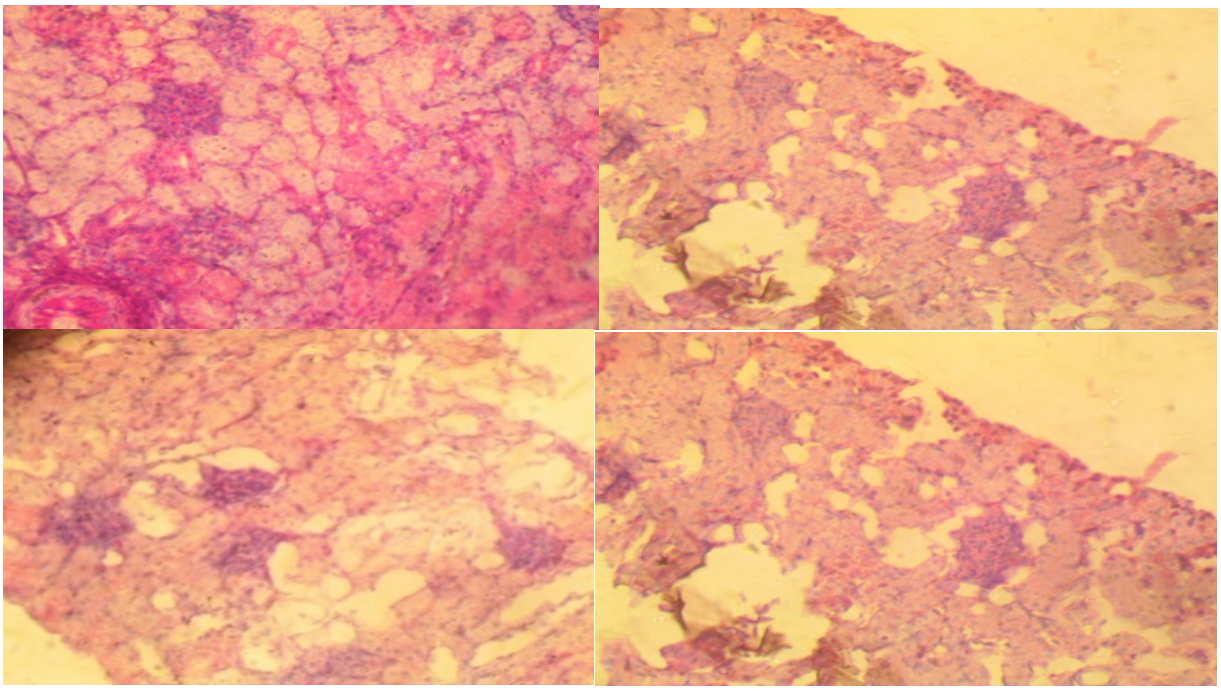


CONTROL DIET GROUP

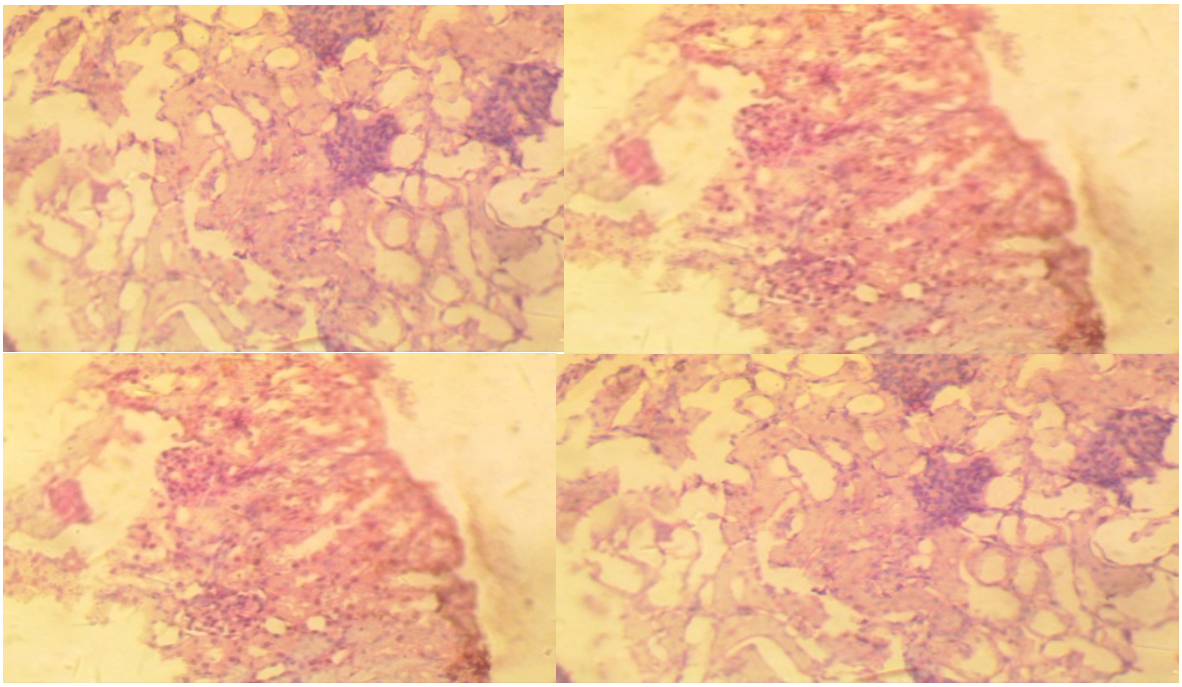
Plate 4.5: Histopathology slides of rats'small intestines



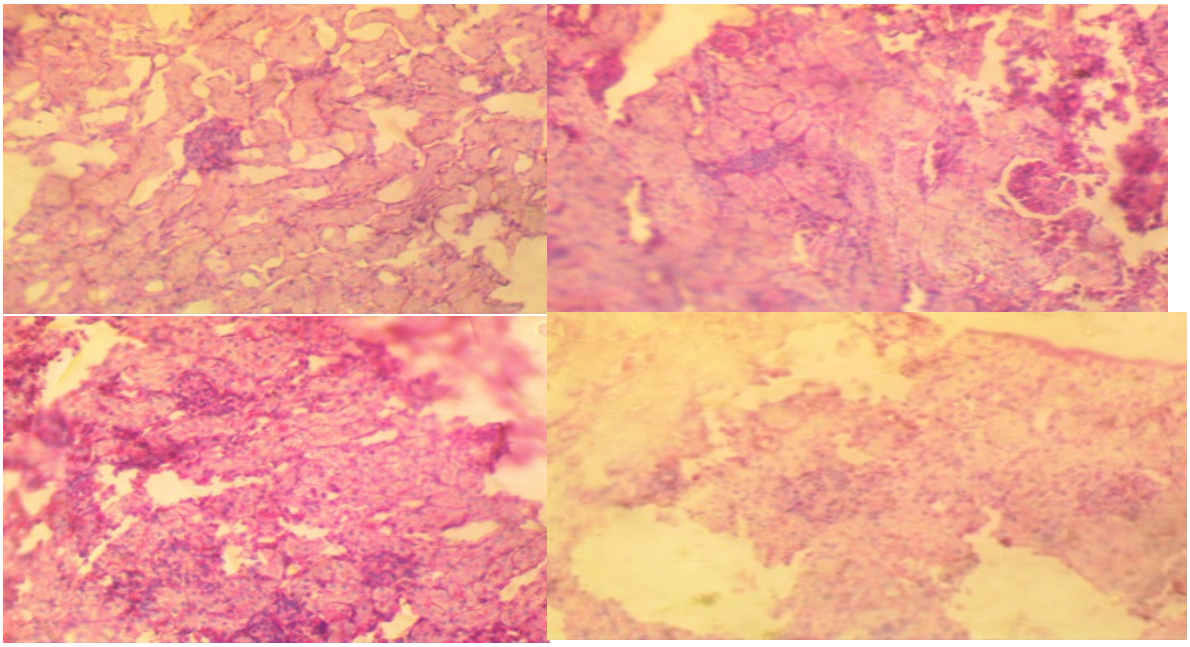
BASAL DIET GROUP



SHAKI *Cirina forda*DIET GROUP

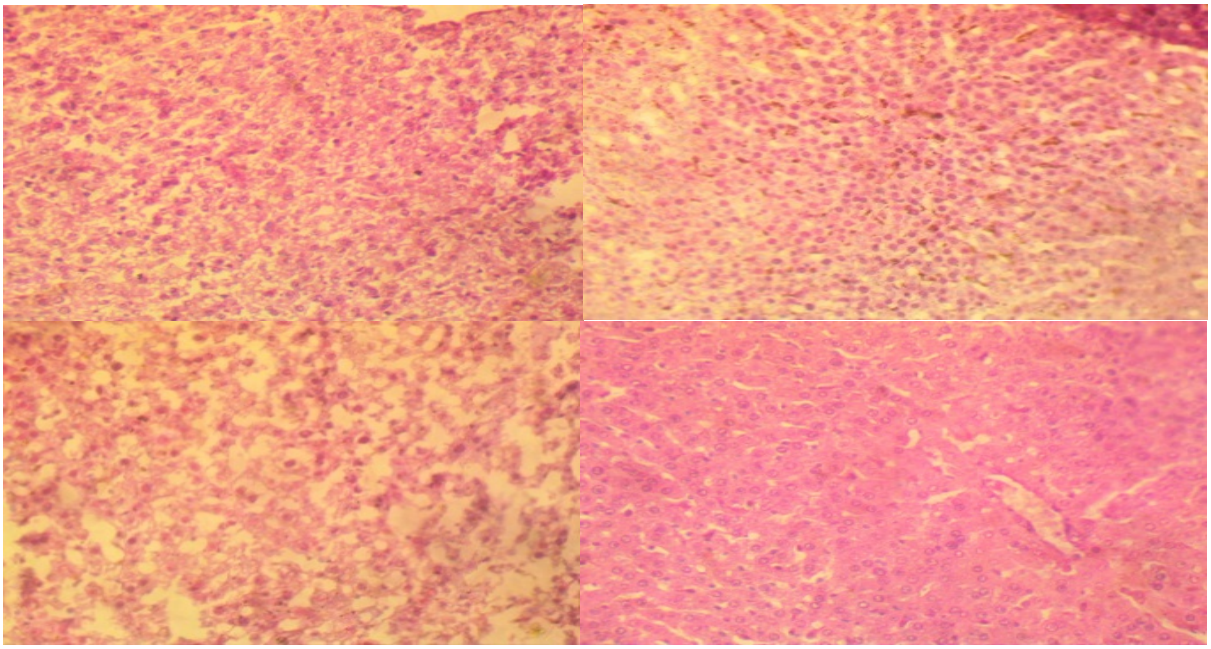


GHANA *Cirina forda*DIET GROUP

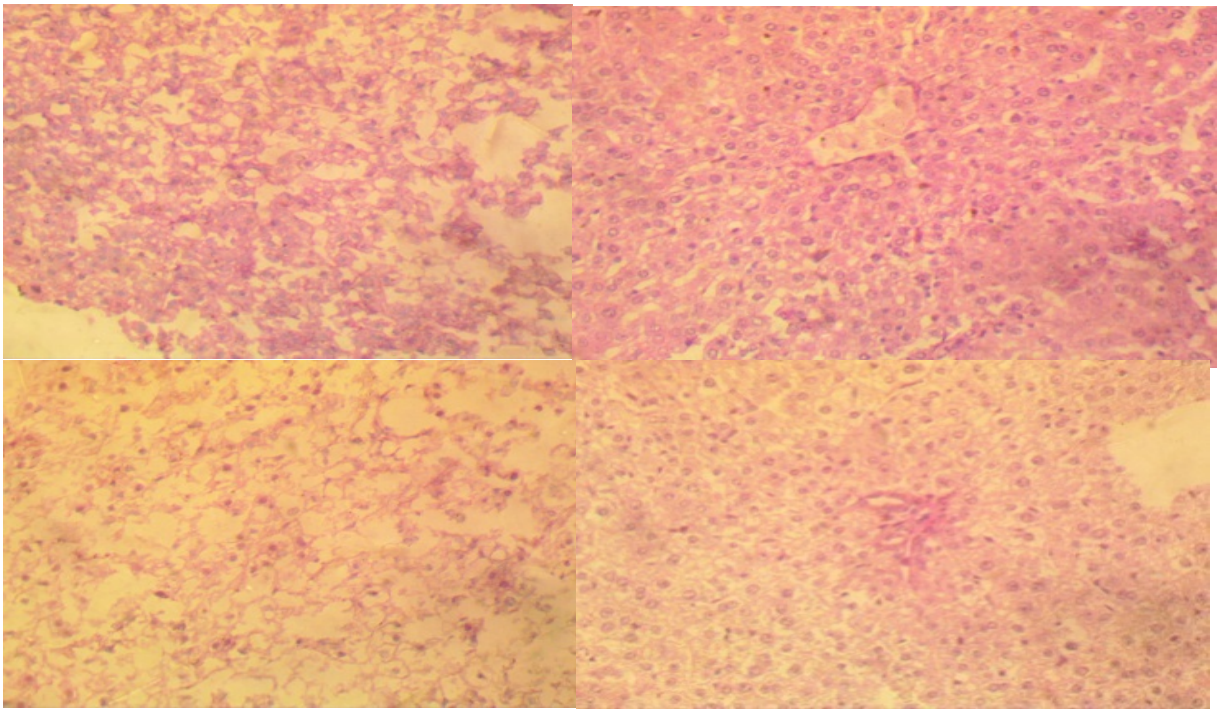


CONTROL DIET GROUP

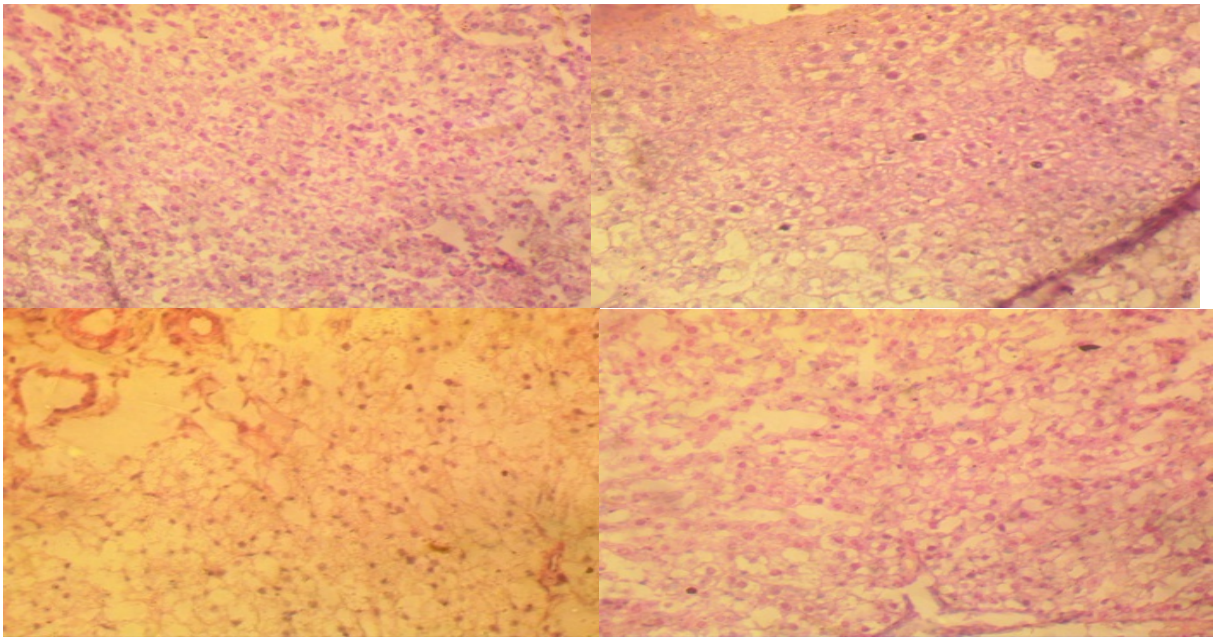
Plate4.6: Histopathology slides of the rats' kidneys



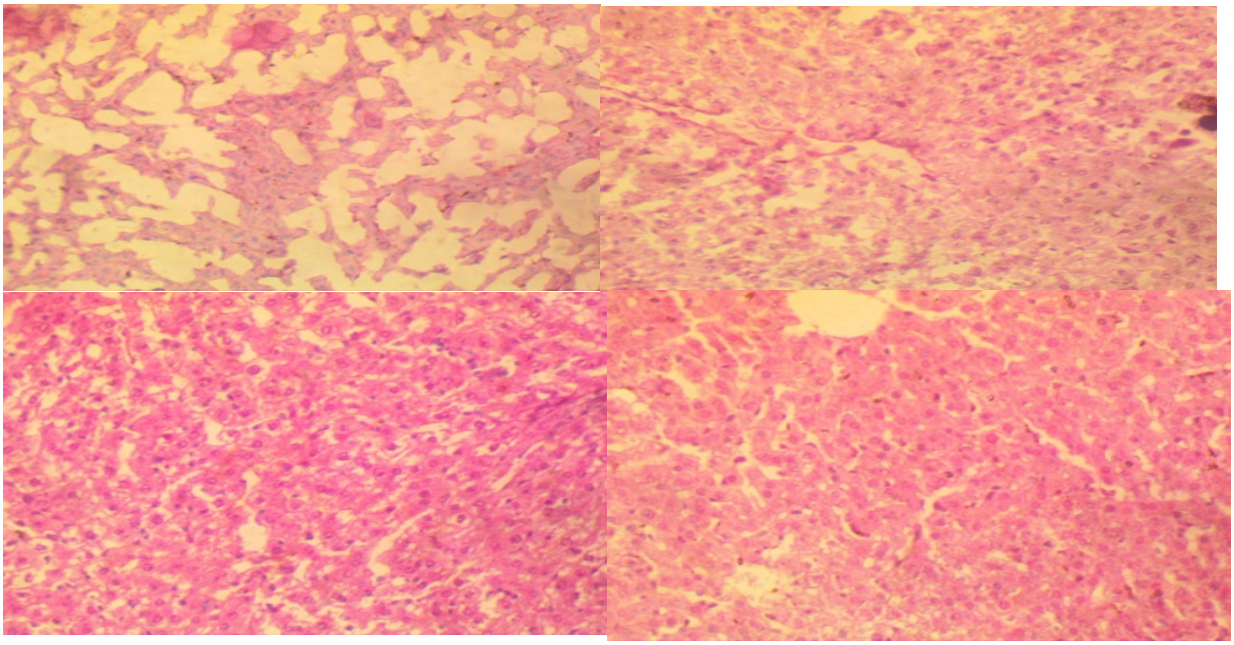
BASAL DIET GROUP



SHAKI *Cirina forda*DIET GROUP



GHANA *Cirina forda*DIET GROUP



CONTROL DIET GROUP

Plate 4.7: Histopathology slides of the rats' livers

Specific Objective 5

To conduct sensory evaluation and determine nutrient composition of *Cirina forda* larvaeenriched vegetable soups

4.28 Sensory Evaluation of vegetable soups

In table 4.28(a), all samples were accepted by the panellists, as none of them was rejected or scored below average. Overall, the control vegetable soup (sample B) was the least accepted for colour, taste, texture, aroma and overall acceptability. The two *C. forda* larva-enriched vegetable soups (samples C and E) were more relished compared with the plain vegetable soup (sample B) and *Egusi* + *C. forda* soup (Sample D). The vegetable + *Egusi* + *C. forda* larva soup (Sample E) was the most acceptable for colour, taste, aroma and general acceptability; while vegetable soup + *C. forda* larva sample (sample C) was the most accepted for texture.

Table 4.28(b) showed that significant differences between the plain vegetable soup (sample B) and vegetable soup enriched with *C. forda* larva (sample C) in all the parameters assessed, the *C. forda* larva-enriched vegetable soup scoring higher ($p<0.05$). *Egusi* soup enriched with *C. forda* larva soup significantly scored higher than *Egusi* soup in taste, colour, and aroma ($p<0.05$); while there was no significant difference in their texture and overall acceptability ($p>0.05$). There was no significant overall acceptability of *C. forda* larva – enriched vegetable soups and *Egusi* vegetable soup ($p>0.05$). The *Egusi* soup enriched with *C. forda* larva scored highest in all parameters assessed.

4.29 Proximate composition of *Cirina forda* and vegetable soups

In the proximate composition of *C. forda* and the four vegetable sample has shown in table 4.30 reported the dry *C. forda* sample was high in crude protein (54%) and gross energy content, high in fat, ash dietary fibre, moderate in carbohydrate but low in moisture content. The Vegetable soup was high in moisture (77.14%) and ash (1.35%) content, high in dietary fibre (11.62%) moderate in crude protein (12.4%) but low in carbohydrate content (4.53%) addition of ground melon seed (*Egusi*) to the vegetable soup (sample D) lowered the moisture content (73.04%) an increase crude protein (14.88%) dietary fibre (12.89%) ash (1.42%), carbohydrates (5.3%), and

gross energy content (153.05 Kcal.) of the vegetable significantly ($p<0.05$). Also, the addition of *C. forda* larva to the vegetable soup (sample B) and *Egusi* Soup (sample D) respectively led to the significant increase in most parameter with significant reduction in the moisture content of enriched vegetable soup samples (samples C and E) ($p<0.05$).

4.30 Mineral content of *C. forda* and vegetable soups

In table 4.31, *C. forda* larva contained a substantial amount of sodium, potassium, calcium, magnesium, phosphorus, with potassium being the most abundant followed by sodium, magnesium, calcium and phosphorus. It is also high in iron content compared with other animal sources. However, the Larvae was low in zinc and copper and low in manganese. The addition of *Egusi* to the vegetable soup (sample C) significantly increase its mineral content additional *C. forda* to the vegetable and *Egusi soup* (sample D) increase the sodium, potassium, iron, zinc and copper significantly ($p<0.05$), while it decreased the calcium, magnesium and phosphorus content significantly ($p<0.05$).

The vegetable soup (sample B) was rich in magnesium, phosphorus and calcium, and high in potassium and sodium content. *Egusi soup* (sample D) increase the nutrient content of the soup also the addition of the insect larva to both (sample B) and (Sample D). sample C and E enriched with *C. forda* significantly increase sodium, potassium, iron, zinc and copper of the enriched vegetable soup.

4.31 Vitamin content of *C. forda* and vegetable soup

Table 4.32 showed the vitamin composition of *C. forda* larva to contain a substantial amount of vitamins, especially vitamins B_{12} and C. The larva was low in water-soluble. The vegetable soup B was low in vitamin A (0.87), B_1 (0.04), B_2 and B_3 but high in B_6, B_{12} and *C. C. forda* enriched vegetable soups (C and E) has a significant increase in the vitamin content of the vegetable soup ($p<0.5$).

4.32 Antinutrients level of *C. forda* larva and vegetable soups

The phytate and trypsin inhibitor content of *C. forda* larva were negligible, while oxalate, tannin and saponin were not detectable as shown in Table 4.33. However, there was a significant difference ($p<0.05$) in the trypsin inhibitor content of the samples ($p<0.05$) with the *C. forda* enriched vegetable soup having a higher value. So enriched vegetable with *Egusi* soup has the highest value of trypsin inhibitor. The level of trypsin inhibitor in the enriched vegetable soups was very low and cannot cause any significant reduction in the bioavailability of protein in the soup.

4.33 Amino acid profiles of *C. forda* larva

The *C. forda* larva has a good amount of essential amino acids such as lysine, threonine, phenylalanine, leucine, isoleucine, valine, tryptophan and cysteine as well as dispensable amino acids such as glutamic acid, histidine, arginine, aspartic acid and glycine among others, hence, its protein content is of high quality (Table 4.34)

4.34Fatty acid profiles of *C. forda* larva

Tale 4.35 showed that *C. forda* larva is rich in linolenic acid, (poly-unsaturated acid), and high in oleic acid (monosaturated) stearic and palmitic acids among others. Linolenic, stearic, are very much abundant in the fat of the larva. Unsaturated fatty acids constituted 52.27% of the total fatty acid in the larvae and 12.15% of this was monounsaturated fatty acid (MUFA).

Table 4.24(a): Sensory evaluation of the soup samples

Parameter	B: Vegetable Soup	C: Vegetable + *Cirina forda*	D: Vegetable + *Egusi* Soup	E: Vegetable + Egusi + *C.forda*
Taste	6.40±2.53[a]	8.03±1.07[b]	7.70±1.32[b]	8.60±0.67[b]
Color	6.13±2.73[a]	7.77±1.43[b]	7.43±1.57[b]	8.13±0.87[b]
Aroma	6.03±2.63[a]	7.73±1.51[b]	7.40±1.50[b]	8.13±0.82[b]
Texture	6.33±2.45[a]	7.60±1.19[b]	7.17±1.62[b]	7.47±1.46[b]
Overall Acceptability	6.13±2.78[a]	7.87±1.07[b]	7.67±1.40[b]	8.20±1.24[b]

**Values are mean ± standard deviation of 30 panellists* (n = 30)

***Values with similar alphabets along the same row are insignificantly different (p>0.05)

Table 4.24(b): Sensory evaluation of the soup samples(Enriched vegetables versus Controls)

Parameter	B: Vegetable Soup	C: Vegetable + *Cirina forda larva*	Sig.	D: Vegetable + *Egusi* Soup	E: Vegetable + Egusi + *C.forda*	Sig.
Taste	6.40±2.53[a]	8.03±1.07[b]	**0.002**	7.70±1.32[b]	8.60±0.67[b]	**0.02**
Colour	6.13±2.73[a]	7.77±1.43[b]	**0.005**	7.43±1.57[b]	8.13±0.87[b]	**0.03**
Aroma	6.03±2.63[a]	7.73±1.51[b]	**0.003**	7.40±1.50[b]	8.13±0.82[b]	**0.02**
Texture	6.33±2.45[a]	7.60±1.19[b]	**0.014**	7.17±1.62[b]	7.47±1.46[b]	**0.45**
Overall Acceptability	6.13±2.78[a]	7.87±1.07[b]	**0.002**	7.67±1.40[b]	8.20±1.24[b]	**0.12**

**Values are mean ± standard deviation of 30 panellists* (n = 30)

**The values are significantly different from each other at* (*p<0.05*).

Table 4.25: Proximate composition of *Cirina forda*and vegetable soup samples (g/100g)*

Parameter	A: *Cirina forda* larva	B: *Vegetable Soup*	C: Vegetable Soup + *C. forda* larva	D: Vegetable + Egusi Soup	E: Vegetable + *Egusi* + *C. forda larva soup*
Moisture	3.98±0.03[a]	77.14±0.02[b]	68.90±0.05[c]	73.04±0.03[d]	59.78±0.03[e]
Crude Protein	54.38±0.10[a]	12.11±0.20[b]	18.68±0.10 [c]	14.88±0.10[d]	23.68±0.10[e]
Crude Fat	16.81±0.03[a]	2.45±0.02[b]	3.26±0.02[c]	2.45±0.03[b]	3.79±0.04[d]
Crude Fibre	0.55±0.02[a]	2.42±0.03[b]	2.19±0.04[c]	2.61±0.03[d]	2.35±0.02[b]
Ash	1.78±0.03[e]	1.35±0.03[d]	1.46±0.02[c]	1.42±0.03[b]	1.61±0.03[a]
Carbohydrate	22.50±0.05[a]	4.53±0.22[b]	5.51±0.14[c]	5.64±0.16[d]	8.79±0.09[e]
GrossEnergy (kcal/)	492.05±0.35[a]	132.89±2.52[b]	173.74±3.00[c]	153.05±1.52[d]	216.23±3.51[e]
Dietary Fibre	8.86±0.02[a]	11.62±0.03[b]	14.57±0.34[c]	12.89±0.03[d]	15.81±0.02[e]

**Values are the mean and standard deviation of three determinations.*

**Values with similar alphabets along the same row are insignificantly different (p>0.05)

Table 4.26: Mineral Composition of *Cirina forda* and vegetable soup samples (mg/100g)*

Parameter	A *C. forda. Larva*	B *Vegetable Soup*	C Vegetable Soup + *C. forda* larva	D Vegetable + Egusi Soup	E Vegetable + *Egusi* + *C. forda larva soup*
Sodium	290.00±2.00[a]	166.67±2.52[b]	195.33±3.01[c]	182.0±3.61[d]	220.67±2.52[e]
Pottassium	623.67±3.06[a]	195.33±3.06[b]	309.67±2.08[c]	208.67±2.51[d]	327.67±3.06[e]
Calcium	262.0±3.00[a]	260.67±2.52[a]	261.33±3.06[b]	325.0±3.00[c]	330.67±3.06[d]
Magnesium	278.67±3.51[a]	359.33±2.52[b]	340.67±2.52[c]	336.67±2.52[d]	323.67±3.06[e]
Phosphorus	146.67±1.53[a]	286.67±2.52[b]	261.0±3.61[c]	301.33±3.06[d]	278.67±3.05[e]
Iron	8.94±0.02[a]	2.80±0.01[b]	3.36±0.01[c]	3.13±0.01[d]	3.96±0.01[e]
Copper	0.33±0.02[a]	0.13±0.00[b]	0.17±0.01[c]	0.15±0.01[c]	0.19±0.00[d]
Zinc	1.39±0.03[a]	0.27±0.01[b]	0.43±0.00[c]	0.28±0.00[b]	0.46±0.00[c]
Mn	0.001±0.00[a]	0.001±0.00[a]	0.001±0.00[a]	0.001±0.00[a]	0.001±0.00[a]

**Values are the mean and standard deviation of three determinations.*

**Values with similar alphabets along the same row are insignificantly different($p>0.05$)

Table 4.27: Vitamin composition of *Cirina forda* and vegetable soup samples (mg/100g)*

Parameter	A: *C. forda. Larva*	B: *Vegetable Soup*	C: Vegetable Soup + *C. forda* larva	D: Vegetable + Egusi Soup	E: Vegetable + *Egusi* + *C. forda larva soup*
Vitamin A (µg/)	0.33±0.02[a]	0.87±0.02[b]	1.25±0.02[c]	1.07±0.03[d]	1.39±0.03[e]
Vitamin B_1	0.19±0.02[a]	0.04±0.01[b]	0.13±0.02[c]	0.11±0.03[d]	0.19±0.04[a]
Vitamin B_2	0.37±0.01[a]	0.09±0.02[b]	0.15±0.01[c]	0.14±0.02[c]	0.21±0.02[d]
VitaminB_3	0.62±0.03[a]	0.71±0.02[b]	0.91±0.03[c]	0.91±0.03[c]	1.08±0.03[d]
VitaminB_6	0.46±0.02[a]	2.11±0.03[b]	2.29±0.03[c]	2.26±0.02[c]	2.45±0.03[d]
Vitamin B_{12} (µg/)	3.53±0.02[a]	17.01±0.03[b]	13.52±0.04[c]	14.57±0.05[d]	11.94±0.03[e]
Vitamin C	19.24±0.03[a]	15.23±0.03[b]	28.75±0.02[c]	18.62±0.04[d]	31.81±0.03[e]
Vitamin E (µg/)	1.89±0.03[a]	3.01±0.04[b]	4.66±0.41[c]	3.79±0.05[d]	5.27±0.02[e]

**Values are the mean and standard deviation of three determinations.*

**Values with similar alphabets along the same row are insignificantly different ($p>0.05$)

Table 4.28: Antinutrient content of *Cirina forda* and vegetable soups (mg/100g)*

Parameter	A: *C. forda. Larva*	B: *Vegetable Soup*	C: Vegetable Soup + *C. forda* larva	D: Vegetable + Egusi Soup	E: Vegetable + *Egusi* + *C. forda larva soup*
Phytate	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Oxalate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.01±0.00[a]	0.00±0.00[a]
Tannin	0.00±0.00[a]	0.01± 0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Saponin	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.01±0.00[a]	0.00±0.00[a]
Trypsin Inhibitor	0.70±0.03[e]	0.91±0.03[d]	1.30±0.02[c]	1.08±0.03[b]	1.45±0.02[a]

**Values are the mean and standard deviation of three determinations.*

**Values with similar alphabets along the same row are insignificantly different ($p>0.05$)

Table 4.29: Amino Acid content of *Cirina forda* larva

Amino Acids	Composition (%)
Alanine, Ala	3.72
Arginine, Arg	2.85
Aspartic Acid, Asp	7.65
Cysteine, Cys	1.87
Glutamic Acid, Glu	13.33
Glycine, G	5.77
Histidine, His	3.54
Isoleucine, Ile	5.37
Leucine, Leu	5.77
Lysine, Lys	19.96
Methionine, Met	2.19
Phenylalanine, Phe	4.47
Proline, Pro	3.36
Threonine, Thr	8.09
Tryptophan, Trp	1.64
Tyrosine, Tyr	5.10
Cystine,	0.67
Serine, Ser	0.24
Valine, Val	2.56

Table 4.30: Fatty acid composition of *Cirina forda* Larva

Fatty Acids	Composition (%)
Arachidonic	0.33
Lauric	3.38
Linoleic	7.07
Linolenic	33.06
Margaric	0.03
Myristic	0.63
Oleic	11.35
Palmitic	16.38
Palmitoleic	0.43
Stearic	27.16

Specific Objective 6

To assess the effect of storage conditions on the shelf-life of dried *Cirina forda* larva

4.35 Microbiological analysis of *Cirina forda*samples

Table 4.36 showed the major microflora obtained in the samples after storage were *Bacillus* spp., *Rhizopus* spp., *Aspergillus* spp., and yeasts. The total heterotrophic count of samples increased with an increase in the number of storage days. At 90 days of storage, the refrigerated Ghana sample, GR (19.2×10^5) had the lowest heterotrophic count, while the ambient stored Ghana sample, GAN (33.7×10^5) had the highest. After 180days of storage, the refrigerated Ghana sample, GR (25.3×10^5) had the lowest while the ambient stored in plastic-container, SAP (39.2×10^5) had the highest heterotrophic count.

4.36 Proximate composition of *Cirina forda*stored for 90 days

Table 4.37 showed that the Moisture, protein, fat, gross energy, and dietary fibre (but not crude fibre and carbohydrate contents of samples GR and SA as well as ash contents of samples SR and GA) significantly differed ($p<0.05$). Also, the proximate nutrient composition of the refrigerated prototypes was higher than those stored under ambient temperature.

4.37 Mineral composition of *Cirina forda*stored for 90 days

Table 4:38 showed the Mineral composition of the samples (except in samples SR and GA) differed significantly from each other ($p<0.05$) and refrigerated samples presented higher mineral contents than those stored under ambient temperature.

4.38 Vitamin composition of *Cirina forda*stored for 90 days

Table 4:39 showed that most vitamin contents of the samples differed significantly with an insignificant difference ($P>0.05$) in vitamins A, B_2, B_6 of samples SR and GA as well as B_1, and B_6 of samples GR and SA. However, refrigerated samples presented higher values than those stored at ambient temperature.

Table 4.31: Microbiological analysis of *Cirina forda*samples (cfu/g)

Sample Code	Total Heterotrophic count ($\times 10^5$)		Major microflora obtained
	Day 90	Day 180	
GR	19.2	25.3	*Bacillus* sp., *Rhizopus* spp.
SAP	32.6	39.2	Yeasts, *Bacillus* spp.
GAN	33.7	*ND	*Bacillus* spp., *Rhizopus* spp.
SR	20.5	26.2	*Bacillus* spp., *Rhizopus* spp.
GAP	22.6	31.5	*Aspergillus* sp., *Bacillus*spp., Yeasts
SAN	23.3	*ND	*Bacillus* spp., *Rhizopus* spp.

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.32: Proximate composition of *Cirina forda*stored for 90 days (g/100g)

Parameter	SR	GR	SA	GA
Moisture	4.92 ± 0.03[a]	3.78 ± 0.03[b]	7.76 ± 0.02[c]	5.47 ± 0.03[d]
Protein	51.28 ± 0.11[a]	53.18 ± 0.09[b]	50.28 ± 0.11[c]	52.37 ± 0.11[d]
Fat	16.58 ± 0.03[a]	17.09 ± 0.02[b]	16.35 ± 0.03[c]	16.86 ± 0.02[d]
Crude Fibre	0.78 ± 0.03[a]	0.65 ± 0.02[b]	0.62 ± 0.03[b]	0.48 ± 0.03[c]
Ash	2.47± 0.02[a]	2.67 ± 0.03[b]	2.31 ± 0.02[c]	2.53 ± 0.02[a]
Carbohydrate	23.97 ± 0.09[a]	22.64 ± 0.05[b]	22.68 ± 0.06[b]	22.29 ± 0.10[c]
Gross Energy	441.63 ± 0.15[a]	457.83 ± 0.25[b]	440.13 ± 0.06[c]	456.93 ± 0.25[d]
Dietary Fibre	8.35 ± 0.03[a]	9.09 ± 0.02[b]	8.19 ± 0.02[c]	8.87 ± 0.02[d]

Values are the mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$)

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.33: Mineral composition of *Cirina forda*stored for 90 days (mg per 100g)

Parameter	SR	GR	SA	GA
Sodium	285.33 ± 2.08[a]	298.0 ± 3.61[b]	269.67 ± 3.06[c]	279.33 ± 3.51[a]
Potassium	618.0± 3.00[a]	643.0 ± 2.00[b]	600.33 ±3.06[c]	629.0 ± 2.00[a]
Calcium	261.0 ±3.00[a]	276.33± 2.52[b]	248.33± 3.06[c]	263.00± 2.00[a]
Phosphorus	148.33± 2.52[a]	156.67 ± 2.52[b]	137.00 ±1.73[c]	143.0 ± 1.73a[c]
Magnesium	281.67±2.52[a]	292.33 ± 1.15[b]	269.0± 2.00[c]	278.33± 2.52[a]
Iron	8.45 ± 0.03[a]	9.18 ± 0.03[b]	8.28 ± 0.04[c]	8.99 ± 0.03[d]
Copper	0.27 ± 0.02[a]	0.36 ± 0.01[b]	0.19 ± 0.01[c]	0.25 ± 0.02[a]
Zinc	1.34 ± 0.02[a]	1.47± 0.01[b]	1.20 ± 0.02[c]	1.30 ± 0.02[a]
Manganese	0.001 ± 0.00[a]	0.001 ±0.00[a]	0.001 ± 0.00[a]	0.001 ± 0.00[a]
Selenium	394.39± 0.02[a]	403.79 ±0.03[b]	394.28± 0.01[c]	403.58 ± 0.03[d]

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$).

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.34: Vitamin composition of *Cirina forda* stored for 90 days. (mg per 100g)

Parameter	SR	GR	SA	GA
Vitamin A	0.25 ± 0.02^{a}	0.36 ± 0.02^{b}	0.16 ± 0.02^{c}	0.26 ± 0.02^{a}
Vitamin B1	0.34 ± 0.02^{a}	0.23 ± 0.02^{b}	0.24 ± 0.02^{b}	0.13 ± 0.02^{c}
Vitamin B2	0.34 ± 0.02^{a}	0.42 ± 0.02^{b}	0.23 ± 0.03^{c}	0.31 ± 0.03^{a}
Vitamin B3	0.86 ± 0.03^{a}	0.68 ± 0.03^{b}	0.73 ± 0.02^{b}	0.57 ± 0.02^{c}
Vitamin B6	0.37 ± 0.02^{a}	0.51 ± 0.02^{b}	0.29 ± 0.02^{c}	0.39 ± 0.03^{a}
Vitamin B12	3.14 ± 0.03^{a}	3.45 ± 0.02^{b}	3.03 ± 0.02^{c}	3.31 ± 0.02^{d}
Vitamin C	18.24 ± 0.01^{a}	19.29 ± 0.03^{b}	18.12 ± 0.03^{c}	19.18 ± 0.03^{d}

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$).

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

4.39 Antinutrient content in *Cirina forda* stored for 90 days

Table 4:40 showed that the Anti-nutritional composition of the samples (except trypsin inhibitor) differed insignificantly ($p>0.05$).

4.40 Proximate composition of *Cirina forda* stored for 180 days

Table 4.41 showed that the Proximate nutrient composition of the samples significantly differed ($p<0.05$) across all samples with the refrigerated sample from Ghana (GR) having higher proximate nutrients than the Saki refrigerated sample (SR).

4.41 Mineral Composition of *Cirina forda* Stored for 180 days

Table 4.42 showed that the mineral composition of samples differed significantly ($p<0.05$), with Ghana refrigerated sample (GR) having higher mineral content than the Saki refrigerated sample (SR).

4.42 Vitamin composition of *Cirina forda* stored for 180 days

Table 4.43 showed that the vitamin composition of samples differed significantly ($p<0.05$), and Ghana refrigerated sample (GR) had higher vitamin content than the Saki refrigerated sample (SR) except for vitamins B_1, and B_6.

4.43 Antinutrient Content of *Cirina forda* Stored for 180 days

Table 4.44 showed that the anti-nutritional composition of the samples (except for trypsin inhibitor) differed insignificantly ($p>0.05$).

Table 4.35: Antinutrient content in*Cirina forda*stored for 90 days (mg/100g)

Parameter	SR	GR	SA	GA
Phytate	0.00 ±0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Oxalate	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Tannin	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Saponin	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Trypsin Inhibitor	0.77 ±0.02[a]	0.61 ±0.02[b]	0.66 ±0.02[b]	0.48 ± 0.03[c]

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different (p>0.05)

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.36: Proximate composition of *Cirina forda*stored for 180 days (g/100g)

Parameter	Sample SR	Sample GR
Moisture	6.05 ± 0.03^{a}	4.92 ± 0.03^{b}
Protein	50.71 ± 0.16^{a}	52.88 ± 0.10^{b}
Fat	16.51 ± 0.04^{a}	16.95 ± 0.03^{b}
C.Fibre	0.69 ± 0.04^{a}	0.57 ± 0.03^{b}
Ash	2.41 ± 0.03^{a}	2.59 ± 0.02^{b}
Carbohydrate	23.63 ± 0.16^{a}	22.09 ± 0.11^{b}
Gross Energy	440.87 ± 0.25^{a}	457.83 ± 0.25^{b}
D.Fibre	8.25 ± 0.03^{a}	9.00 ± 0.04^{b}

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$).

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.37: Mineral composition of *Cirina forda*stored for 180 days (mg/100g)

Parameter	Sample SR	Sample GR
Sodium	280.67 ± 2.52^{a}	289.68 ± 2.08^{b}
Potassium	610.67 ± 2.52^{a}	636.0 ± 2.00^{b}
Calcium	255.33 ± 2.52^{a}	270.0 ± 1.73^{b}
Phosphorus	144.0 ± 2.00^{a}	149.33 ± 2.52^{a}
Magnesium	275.67 ± 252^{a}	287.00 ± 1.73^{b}
Iron	8.34 ± 0.02^{a}	9.06 ± 0.02^{b}
Copper	0.22 ± 0.01^{a}	0.31 ± 0.02^{b}
Zinc	1.29 ± 0.02^{a}	1.36 ± 0.02^{b}
Manganese	0.001 ± 0.00^{a}	0.001 ± 0.00^{b}
Selenium (µg/)	394.33 ± 0.02^{a}	403.69 ± 0.04^{b}

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$)

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.38: Vitamin composition of *Cirina forda*stored for 180 days (mg/100g)

Parameter	Sample SR	Sample GR
Vitamin A (µg/)	0.20 ± 0.01^{a}	0.33 ± 0.02^{b}
Vitamin B6	0.33 ± 0.02^{a}	0.45 ± 0.02^{b}
Vitamin B3	0.81 ± 0.03^{a}	0.63 ± 0.03^{b}
Vitamin B1	0.30 ± 0.02^{a}	0.18 ± 0.01^{b}
Vitamin B2	0.30 ± 0.01^{a}	0.39 ± 0.02^{b}
Vitamin B12 (µg/)	3.08 ± 0.03^{a}	3.39 ± 0.03^{b}
Vitamin C	18.21 ± 0.03^{a}	19.25 ± 0.03^{b}
Vitamin E (µg/)	1.82 ± 0.04^{a}	1.93 ± 0.02^{b}

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different ($p>0.05$)

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

Table 4.39: Antinutrient contentin*Cirina forda* stored for 180 days (mg/100g)

Parameter	Sample SR	Sample GR
Phytate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Oxalate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Tannin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Saponin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Trypsin Inhibitor	0.72 ± 0.01^{a}	0.55 ± 0.02^{b}

Values are mean and standard deviation of three determinations.

Values with similar alphabets along the same row are insignificantly different*(p>0.05).*

GAN= Ghana *Cirina forda* stored in nylon at ambient temperature; SAP= Saki *Cirina forda* stored in plastic; GR= Ghana *Cirina forda* refrigerated; SAN= Saki *Cirina forda* stored in nylon at ambient temperature; GAP= Ghana *Cirina forda* stored in a plastic container; SR= Saki *Cirina forda* refrigerated.

*ND- Not Detected

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 *Cirina forda* larva consumption pattern

Consumption of *Cirina forda* cut across all age groups, sex, ethnic group, religion, marital status, educational level, occupation, and financial status of the respondents in the two (2) selected Local Governments Area (Table 4.1). The social demographic characteristics are similar to those reported by Mandistera *et al.,* (2018). Socio-demographic status has nothing to do with the consumption of the insect larvae as most of them reported that they were aware of its existence and had all consumed it before at a point in time since childhood. It is usually referred to as "monimoni" in the local language by most of the respondents, while few people referred to it as "ikanni".

Cirina forda was consumed by most people on weekly basis and few consumed it daily. For most respondent that had consumed it in the last two years scarcity, marriage and civilization were reasons for their non-consumption while some did not because of the fear of consuming poisonous species. Non-consumption of the larvae due to civilization as reported by some of the respondents in the study corroborates the finding of Ebenebe *et al.,*(2015), who reported that insect-eating was greatly associated with poverty, as the rich/elites drop the habit for the poor and the illiterates in the rural communities. Also, a common belief is that traditional foods like edible insect, are considered to be primitive and are not acceptable by Western communities Mandistera *et al.,*(2018).

The form in which the larvae are been consumed was either roasted or fried, boiled, fried, or in powder. The most preferred form of *Cirinaforda* was condiments in soup such as Efo riro, Tomatoes and Egunsi Soup (Adepoju and Daboh, 2020). This also supports the statement of Fasoranti and Ajiboye (1993) that insect is widely used as an ingredient in the vegetable soup a declined in the rate of consumption of *Cirina forda* larvae reported by the respondents was attributed to different reasons ranging from Civilization, seasonality, price and income to fear of

consuming the poisonous species. The reason is in line with the report of other researchers of reduced consumption of edible insect namely – the adoption of Western foods (Dube*et al.,* 2013; Looy*et al.,*2014*;* Mlcek *et al.,* 2014), decreased knowledge of preparation practice Riggi *et al.,*(2016), unavailability of edible insect Looy *et. al* (2014), uncontrolled harvesting Rasmos-Elorduy, (2006), and loss of habitats, leading to the extinction of some species Duba *et al.,*(2013) Mayer-Rochow and Chakravorty, (2013)

Cirina forda was reported by most of the respondents not to be available all year round, probably due to its seasonality. This supports the report of Odeyemi and Fasoranti (2000) and Agbidye and Nongo (2009) which stated that the larvae were usually collected during the rainy season between June and August. Also, Latham, (2001) reported that cultivation and bush burning reduces the availability of edible caterpillars. Food habits/customs, flavour and taste, nutritional/health-benefits, and economic preferences were the reason attached to the consumption of the larvae. Consumption of the insect as a result of its taste and nutritional benefits by the respondent in this study corroborates the finding of Mandistera, (2018) *et. al*. Obopile and Seeletso, (2013) reported that respondents consume insect for their nutritional value because they perceive insect as high-value nutritious food but do not know the exact nutritional values. This was also observed in this study as most of the respondents reported that they are not aware of the nutritional and health benefit of *Cirina forda.* This call for extensive public enlightenment on the nutritional and health importance and benefits of the edible insect in our community Daboh and Adepoju, (2020). The preference of *Cirina forda* larvae over meat, fish and eggs by some of the respondent was based on the *Cirina forda* been fleshy/boneless, economic reasons, nutritional benefits and taste. The study also shows that one of the major factors limiting the consumption of this insect was availability. This is in line with the findings of other studies which indicated that availability influences preference and consumption of edible insect Mandistera *et al.,* (2018). Age was also identified as a contributing factor to the consumption of the larvae as few of the respondents indicated that it is mostly consumed by adults 35 and above. Studies had shown that there is a tendency for younger populations to abandon the practice of entomophagy due to the Westernization of traditional diets, Vantomme (2015). A few also reported that religious beliefs and traditional beliefs/myth affect the consumption of *Cirina forda* Duba*et al.,*(2013) which stated that entomophagy is forbidden in

some religious practice. However, this is in sharp contrast to the findings in this study asalmost all the respondents (99.8%) stated that religion or traditional/myth did not affect the consumption of *Cirinaforda* or other popular edible insects in the study communities.

5.1.2 Chemical composition of dried *Cirina forda* larvaeand nutrient comparison of the samples from the selected locations

5.1.2.1 Proximate composition and comparison of dried*Cirina forda*larva samples

The moisture content of the dried *Cirina forda* larva samples was found to be low,justifying reports ofBanjo*et al.,* (2006); Osasona and Olaofe (2010) and Igbabul*,* Chiaand Comfort, (2014) who also reported low moisture content for *Cirina forda*. However, the range of the moisture content reported in this study is lower than that of Igbabul, Chia and Comfort (2014). The low moisture content of the larva perhaps is advantageous in keeping quality (shelf life) and preventing microbial contamination. The Ghana samples showed the lowest moisture content of 3.29% compare to 4.01% and 3.98% for Saki and Burkina Faso samples respectively.

The dried *Cirina forda* larva samples showed very high protein content which was in agreement with the range reported by Igbabul *et al.,* (2014) and the values reported by Adepoju and Daboh (2013). Edible insects have been shown to present higher protein reserves, on a mass basis, than other animal and plant foods (Teffo *et al.,* 2007; Alamu *et al.,* 2013). Protein is essentially vital as enzymatic actions, hormonal regulation, haemoglobin production and immunity in the body (Alamu *et al.,* 2013). The Burkina Faso sample recorded the highest values of protein with 54.38% to Ghana sample, 53.4% and Saki, 51.88%. The result of this study showed that irrespective of the locations, *Cirina forda* has substantial high protein values which can help the consumers meet part of their recommended dietary allowance, RDA for protein when consumed.

The crude fat content of the *Cirina forda* larva samples obtained in this study corroborated the findings of Adepoju and Daboh (2013), and greater than the values reported by Osasona and Olaofe (2010), and Igbabul*,* Chiaand Comfort (2014). Adepoju and Ajayi (2016) reported a similar value for *M. bellicosus*. Theobserved difference in the *Cirina forda*fat content in this study can be attributed tothe difference in geographic locations of the samples which can be seen in the recorded values for the Ghana sample with 17.21%, Burkina Faso, 16.81% and Saki with

the lowest value of 16.69% and seasonal variation. However, the amount of fat in the *Cirina forda* larva is beneficial because the rate of susceptibility to rancidity is reduced.

The crude fibre content in this report was lowat the overall mean value of 0.72%. This is expected, as the larva is of animal origin. The value was however comparable to the values reported by Ogunleye (2006), but much lower than the range reported by Igbabul *et al.,* (2014) for crude fibre in *Cirina forda* and *Cirina butyrospermi*. Burkina Faso sample had the lowest crude fibre of 0.55% and the Saki sample with the highest value of 0.86%. Ash content of the dried *Cirina forda* larva samples(Ghana sample, 2.71%, Burkina Faso, 2.585 and Saki, 2.51%) was close to the range of 2.91-3.97% (Igbabul *et al.,* 2014), but lesser than values reported by Akinnawo and Ketiku (2000); Omotosho (2006); and Osasona and Olaofe (2010).The carbohydrate content of the *Cirina forda* larva (Saki sample, 23.92%; Burkina Faso sample, 21.74% and Ghana sample, 21.68%) in this study were higher in values than that reported by Igbabul *et al.,*(2014) and Adepoju and Daboh (2013), but lesser than the value for *M. bellicosus* by Adepoju and Ajayi (2016).

High-protein insect species have been reported to have lower energy content (Bednarova, 2013). However,the gross energy profile of *Cirina forda* was moderately high, given insect nutrient and energy demand (at the larval stage) for growth and development to pupal stage and adulthood is very high. This is in tandem with what was reported by FAO (2004), where higher energy reserves were found in feeding caterpillar flours due to the amount of its protein, fat and carbohydrate contents which were high. The gross energy content of the dry larva samples used in this study which were from Ghana, 458.63Kcal, 456.27Kcal and 442.03Kcal werelower than the 492.31Kcal reported in Cirina butyrospermi by Yapo *et al.,* (2017), but comparable to the 450.7Kcal reported by Adepoju and Ajayi (2016) in *M. bellicosus.*This could be as a result of variance in the nutrient composition of the leaves of the host tree in addition to the geographical location of the insect.

5.1.2.2 Mineral composition and comparison of dried*Cirina forda*larva samples

Cirina forda larvae are good minerals stores. The value obtained for sodium in*Cirina forda* larva samples here weresimilar to the reported values for other edible insects by Ajai, *et al.,* (2013).According to FAO, (2013),2400mg/day of sodium is RDA for a day.It then means that a 100g portion of *Cirina forda* larva would helpsupply about 12% of the daily recommended sodium. Sodiumaids in maintainingextracellular acid-base balance and osmotic pressure in blood and cells (Paiko*,et al.,* 2014). Ghana samples recorded the highest values for almost all the mineral content detected in the *Cirina forda* samples.

Cirina forda larva sampleswere rich in potassium (Ghana sample:309mg/100g, Saki sample:627mg/100g and Burkina Faso sample with a value of 623mg/100g) which werepresent in abundance when compared to all other minerals in *Cirina forda* larva. This finding supports the result of Osasona and Olaofe (2010) where potassium was reported as an abundantly available mineral in *Cirina forda* flour.

Cirina forda larva was high in calcium. The value obtained for calcium in this study were higher than those reported in the literature (Omotosho, 2006; Osasona and Olaofe, 2010). This may be due to the differences in the geographic location of samples. Calcium is an essential constituent necessary for maintenance and normal-functionality of bones and teeth, muscular contraction, the transmission of impulse in the Central Nervous System (CNS) and blood clotting (Yapo *et al.,* 2017, Heaney, 2006).

The content of phosphorous of the larva samples in this study appearedhigher than those reported by Paiko,*et al.,*(2014) but lesser than the 215.54mg/100g reported by Omotosho (2006). Phosphorus is important in the calcification of bones, teeth and nutrient oxidation for the formation of phosphate groups in Adenosine triphosphate (ATP) (Paiko, *et al.,* 2014).

Magnesium was reported to be high in *Cirina forda* larva samples. The values reported in this study were of higher magnesiumcontent higher than those reported byOmotosho (2006) and Osasona and Olaofe (2010). About three hundred enzymatic reactions in the neuromuscular and cardiovascular systems rely on magnesium as a co-factor for protein, carbohydrate, lipids and

nucleic acid syntheses (Chen and Feng, 2002, EFSA 2015a) in addition to the significant roles cell wall integrity and photosynthesis plays (Paiko *et al.,* 2014).

A substantial amount of iron *was found to be in Cirina forda* larva. The desirability of *Cirina forda* larva is unavoidable in humans given their huge iron reserveswhich may likely promote blood enrichment to avoid anaemia.

Copper, zinc, manganese and selenium also occurred in a substantial amount in *Cirina forda* larva, with selenium being the most abundant and manganese the least. The content of copper in the larvawascloseto the value recorded by Ande (2003). The vitality of zinc cannot be underscored in preventing of alopecia, growth retardation, poor appetite, comprised sexual/bone maturation, skin lesions, diarrhoea and defensiveness to infection (FAO/WHO, 2001).

5.1.2.3 Vitamin compositionand comparison of dried*Cirina forda* larva samples

Generally, insects have the record of huge vitamins of both water and fat-soluble. The insect larva contained appreciable amounts of vitamins B_{12}, C and E, with vitamin C reported as the most abundant vitamin. Vitamin C aids in maintaining the flexibility of blood vessels and improved blood arterial circulation (Alamu *et al.,* 2013). Also, vitamins A, C and E are natural antioxidantsthat scavenge for free-radicals, but vitamin B-complex is crucial as co-enzymes in multiple enzymatic systems (Alamu *et al.,* 2013). The result of the vitamins reported in this study for the *Cirina forda* larva samples from the three locations showed that there wasa significant difference ($p<0.05$) with the Ghana sample recorded the highest values for vitamins B_{12}, C and E. The consumption of the Cirina forda larva regularly will help consumers meet their RDAs of these vitamins by not less than 30% on daily basis.

5.1.2.4 Antinutrients content and comparison of dried*Cirina forda* larva samples

The antinutrient content in the insect larva was much lesser than the values reported by Alamu *et al.,* (2013) and Omotosho and Adewale, (2018). Anti-nutrients reduce nutrient bio-availability in the human body. The toxicity of high phytate levels to human health and compromised availability of some nutrients has been well reported by Anuonye *et al.,* (2012). Anti-nutrients reduce solubility and digestibility of protein, as well as impair the bio-availability of calcium,

magnesium, iron and phosphorus by binding them to form an insoluble compound. Also, tannins exert lowered availability of protein by aggressive opposition to promote protein deficiency conditions (Ekop, 2004). The values reported for trypsin inhibitor was the only anti-nutrient in the study that indicated significant difference ($p<0.05$) and will little or no impact on the protein availability or digestibility of this larva upon consumption by the consumers.

5.1.2.5 Amino composition and comparison of dried *Cirina forda* larva samples

The values of the *Cirina forda* larva samples in this work showed that the edible insect contained all the nine essential amino acids, EAAs in substantial amounts for use. Proteinquality is hinged on amino acid composition. *Cirina forda* larva was found to be rich in a wide array of amino acids, most especially the essential amino acid; with lysine, glutamic acid, threonine, aspartic acid, leucine, glycine, isoleucine, tyrosine and phenylalanine being predominant. In tandem with our findings, Yapo *et al.,* (2017) reported abundant amino acid stores in *Cirina butyrospermii*.Amino acids are significant machinery for therapeutic and protein architectural processes; and any deficit in them could hamper these process (Zuraini *et al.,* 2006). Glycine is needed in impulse transmission in the CNS with alanine strengthening immunity architecture to avert the accumulation of toxic materials (Paiko *et al.*, 2014). *Cirina forda* larva protein contains all the beneficial amino acids reserve to complement the availability of essential amino acids in the body.

5.1.2.6 Fatty Acids composition and comparison of dried *Cirina forda* larva samples

The fatty acid profile of the dried insect larva samples wasin linewith the findings of Ande (2003). The result revealed linolenic acid, stearic acid, palmitic, and oleic acid were the most available in the larva. This corroborated the findings of Akinnawo and Ketiku (2000) who reported these fatty acids to be among the most abundant in *Cirina forda* larva.

Linolenic acid, which is the most abundant fatty acid in the larva, is indispensable for retinal operations and nerve architecture (Ande, 2003). Unsaturated fatty acids constitute 51.25%, 52.28%, and 52.67% of the total fatty acids in the larva samples and 39.73%, 40.47%, and 40.89% of these are polyunsaturated fatty acids (PUFA). The ratio of polyunsaturated to saturated fatty acids (which indicates the cholesterol-lowering capacity of a food: 0.2 and 0.8 has

been associated with high and low cholesterol level in addition to a high and reduced risk of coronary heart disorders respectively) of *Cirina forda*larva in this study is 0.9 for all the samples, thus, making it a cholesterol-lowering agent in mitigating the risk of cardiovascular diseases. Interestingly, all three samples for the comparison of the nutrients and anti-nutrients followed the same pattern in terms of having high or low values. They indicated the same progression in values. The higher the value of PUFA and MUFA the better as they aid in the smooth run of the heart and excelling performance of the body arteries and other blood vessels. Consumption of edible insects should be seen as a cheap source of meeting up of RDAs.

5.1.3 Toxicologicalstudyof dried*Cirina forda* larva

For the single-dose oral administration and feeds intake experiments in this study, the result showed that rats were well fed, no clinical signs observed within the twenty-eight days of the experiment, no mortality in the rats' groups and they are morphologically stable before being sacrificed for serum and other biochemical studies. The rats in the experimental groups consumed more feed than those of the control groups. The weight of the rats significantly increased after 14 days-duration of the experiment. The group of rats dosed with *Cirina forda* liquid extract had a higher weight gain than the control group, indicating a high biological value of the larva protein. Also, rats on 80% and 20% *Cirina forda*feed recorded the highest and lowest weight gain respectively.

Relative organ weight differed insignificantly between control and experimental groups, except in liver weights of rats on 80%-*Cirina forda*. This result tallies with the observations of Ekpo (2011). The difference in liver relative weights may be as a result of stress or changes in the relative composition of the tissues due to the test diet. The significant increase in relative liver weight may be attributed to high body weight gain in the 80% *Cirina forda* diet group.

The haematological parameters of the rats were normal and differed insignificantly among control and experimental groups. Similarly, serum chemistry values of rats fed with supplemented feeds differed insignificantly from control except for the Alkaline phosphatase (ALP) level of 80%-*Cirina forda*group which was significantly higher than that of control. This is also similar to the findings of Ekpo (2011) who also observed increases in the alkaline

phosphatase activity in rats fed at various levels of *Imbrasia Belina*larva compared to those fed with casein diets. Alkaline phosphatase, ALP is a liver bio-catalyst present in canalicular and sinusoidal membranes of the liver. The increase in the ALP of the 80%-*Cirina forda*group and the significant increase in its liver weight could be an indication of liver toxicity. This could be since supplementation of *Cirina forda* at 80% might be too high for the rats, and its supplementation at such a high dosage could be hepatotoxic and deleterious to the rat organ. However, the normal range for these enzymes in rats has not been ascertained.

The Urinalysis of the rats single-dose oral administration and feeds intake experiments in this study showed urobilinogen was normal. Bilirubin found in the rats was in low concentration. Blood was not found in the rats' urine samples. This is an indication that consumption of *Cirina forda* larva poses no health risk to the consumers. The phytochemical screening result showed that consumption of this larva poses no threat to health as the phytochemical present in the larva are in low concentration.

There was no statistically significant alteration in the serum protein, iron, calcium, zinc, cholesterol and vitamin A values among control and experimental groups.

The result of the proximate composition of *Cirina forda*powder and the feeds revealed that *Cirina forda* powder had higher protein, fat, ash, and gross energy profiles, while the commercial Topfeed starter mash had the highest crude fibre, carbohydrate, and dietary fibre values. Mineral and vitamin contents (but anti-nutritional composition) of the feeds differed noteworthily.

The phytochemical screening of the *Cirina forda* liquid extract shows alkaloids, tannins, saponin and phenolics to be present in appreciable amount; while phylobatannin, terpenes, flavonoids, steroids and anthraquinones were present in moderate amounts.

During the histopathological examination of the rats in the repeated feeds intake, there was no remarkable lesion notice in slides of the brain cells of the rats and one mild lesion observed in 80% *Cirina forda* inclusion. Mild congestion of the meninges was observed with mild spongiosis for the control feed. The kidney slides of control group rats showed no lesion, mild protein cast at the tubular lumen and basophilic tubule. The kidney for 20% *Cirina forda*inclusion showed a moderate lesion with low hydronephrosis and others with no remarkable lesion, mild cellular

infiltration of the portal area, while 80% of *Cirina forda* diet inclusion group had congestion at the portal area.The histopathological examination in this study is similar to the findings of (Hyeon, *et al.,* 2016).

5.1.4 Bioavailability studyof dried*Cirina forda* larva

The low feed intake of the basal group of animals in this study is due to the lackof protein in the diet, which is an indication that the presence of protein in foods can improve its consumption (Adepoju, 2016). It also correlated with their apparent growth failure/weight loss. The significant weight loss observed among rats on the basal dietwas a confirmation of the non-existence of protein in their diet.

However, rats on experimental diets presented higher weight gained even more than those fed on the control diet despite their lower feed intake. The significant weight gains in the experimental groups indicated that these rats' diets containeda high biological value of protein, which is usually of animal origin, and it is bio-available for rats' growth.

In agreement with (Adepoju, 2016), the higher the Efficiency feed Conversion Index, ECI for the experimental groups led to the significantly higher weight gain in experimental groups compared to the control and basal diet groups. The Protein Energy Ratio (PER) value for the experimental diets was greater than that of the control diet. This is similar to the findings of Ekpo (2011) who found the PER value for *Rhynchophorus phoenicis*diet to be higher than the value obtained for the casein diet.

The serum analysis showed that the values obtained for minerals, vitamin A and total serum protein of rats on control and experimental diets were significantly elevated than those on the basal diet. The contribution of *Cirina forda* to the rat's serum level of these nutrients was an indication that the nutrients were bio-available in rats, and by extension, will be bio-available in humans at the 15% level of inclusion (Sanchez-Muniz *et al.,* 1998).

5.1.5 Sensory evaluation of Vegetable soups

Although, no significant difference was observed in the scoring of sensory attributes of samples C, D and E ($p>0.05$), the enriched samples (C and E) had the highest scoring sensory scores. The exoskeleton of insects has a great influence on the texture. Insects are crunchy and the sounds accompanying their eating resemble the sounds of crackers (Kourimska and Adamkova, 2016). This may explain why Cirina forda enriched vegetable soups were more preferred and scored higher than plain or egunsi vegetable soups. The result of sensory evaluation obtained in which the vegetable soups rated higher in all the parameters is similar to the report of Adepoju and Ugochukwu (2019) in which the vegetable sauce was rated higher than ordinary vegetable soup.

5.1.5.1 Proximate composition of *Cirina forda* and vegetable soups

In table 4.27, the dry *C. forda* larva was low in moisture content (3.98%). The low moisture content obtained for the *C. forda* is in line with the reports of Banjo, *et al.,*(2006) and Osasona and Olaofe (2010). The low moisture content is suggestive of its high keeping quality (long-shelf-life) and supportive of the assertion of the respondents in our consumption survey carried out earlier that the insect larva can be kept for a long period (Daboh and Adepoju, 2020). The low moisture content will prevent microbial contamination. The insect larva containsa high amount of protein. The value obtained for the *Cirina forda* larva protein falls within the range of value reported by Igbabul *et al.,*(2014), similar to 55.41% obtained by (Yapo, *et al.,,* 2017; Adepoju and Daboh, 2013) but lower than 63% reported by Anvo *et al.,* (2016) for *Cirina butyrospermi.* The variation observed in the biochemical composition of the insect larvae maybe due to the host tree because the amount of protein varies between insect species and within the same species depending on the nutritional quality of the leaves of the host tree (Banjo, *et al.,*2006; Yapo *et al.,*2017), the difference in the geographical location where the *C.forda* larva was obtained as well as method of harvesting or processing of the insect larva (Expo, 2011; Womeni *et al.,*2012; Adepoju, 2013).

The crude fat content of the insect larva obtained in this study is within the range of values quoted for insects (10 – 30%) (Durst *et al.,*2010). The value is higher than the values reported by

Osasona and Olaofe (2010) and 15% reported by Anvo *et al.,*(2006), but lower than the 22.21% reported by Ogunleye (2006). The difference in the crude fat content of *C. forda* in this study compared with other studies could be due to the method of processing (Ekpo 2011 *et al.,)* Moderate fat content of the *C. forda* larva is beneficial because it may reduce its rate of susceptibility to rancidity.

However, *C. forda* larva contained a moderate amount of dietary fibre. Dietary fibre helps to regulate the digestive system, aid bowel health and weight management (Rolfe *et al.,* (2009). The value of the ash content of the *Cirina forda* larva sample was less than the range of values (2.91-3.97%) reported by Igbabul, *et al.,*(2014). The gross energy content of the *Cirina forda* sample was very high. This is because at the larval stage, the insect feeds much to derive and store the nutrient and energy needed for growth and development during the pupal stage to the adult stage. The high energy content could also be a result of its high protein, fat and carbohydrate content. The gross energy content in this study is very similar to those of 492.31 kcal reported by Yapo *et al.,*(2017) for *Cirina butryospemi*. The moisture content of the vegetable soup was very high (sample B). The vegetable soup was moderately high in protein content compared with other plant protein (Adepoju and Ugochukwu, 2019). This amount of protein is believed to have been contributed partly by other ingredient used in the preparation of the vegetable soup. The soup was low in fats and carbohydrate despite the addition of palm oil. Leafy vegetables are a poor source of lipids and carbohydrates (Adepoju and Ugochukwun, 2019). However, the soup had a moderate value of ash and gross energy, and high in dietary fibres. Vegetables, generally area good source of dietary fibre. The increase in the dietary fibre of the soup is very beneficial as dietary fibre promotes a healthy bowel function, help to control blood sugar level, lowers blood cholesterol and help in weight management (Rolfe *et al.,*2009).

5.1.5.2 Mineral composition of *Cirina forda* larva and vegetable soups

The sodium content of the larva is within the range reported for other edible insects (Ajai, *et al.,* 2013). The recommended daily allowance of sodium is 2400mg per day (FAO, 2010). Sodium helps in maintaining the proper acid-balance and in controlling osmotic pressure that develops between the blood and cells due to ionic concentration differences (Paiko, *et al.,* 2014).

Potassium was the most abundant of all the minerals in *Cirina forda*larva. This corroborated the findings of Osasona and Olaofe (2010) who reported potassium to be the most abundant mineral in *C. forda*in Cirina forda flour. The high potassium content is highly beneficial because a high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks (Insel, *et al.,* 2007), and it is essential for the functioning of the brain and nerve (Iombor, *et al.,* 2017). The potassium-sodium ratio (K:Na) has frequently been used as a diagnostic tool to identify adrenal insufficiency (Iombor, *et al.,* 2017).

The potassium to sodium ratio in this study is 2.1:1. Hence, the larva could be incorporated into diets for the management of hypertension as high potassium intake has been found to lower blood pressure by antagonizing the effect of sodium.

The Calcium content for the Cirina forda in this study was higher than those reported by (Akinnawo and Ketiku, (2000); Omotoso, (2006) and Ososona and Olaofe, 2010). The observed difference could be due to the differences in the source of the larva coupled with methods of processing. Calcium is an integral component of the skeleton, about 99% of the total body calcium is found in bones and teeth. It plays important role in the development and maintenance of bones and teeth, blood clotting, nerve impulse transmission, and cell metabolism (Heaney, 2017).

The magnesium content of Cirina forda larva was higher than those reported by Akinnawo and Ketiku, (2000), and Omotoso, (2006). The phosphorus content of *C. forda*in this study is higher than the one reported by Paiko, *et al.,* (2014) but lower than 215.54mg/100g reported by Omotoso, (2006). Phosphorus like calcium is also involved in the calcification of bones and teeth.

Cirina forda contained a substantial amount of heme iron. Iron plays an important role as a heme molecule in red blood cells as it permits oxygen transport (WHO, 2006). Since *Cirina forda* larva contained a substantial amount of iron, its inclusion in human diets will be beneficial, and as a blood-building element in anaemic conditions (Rolfe *et al.,* 2009)

Micro-minerals such as copper, zinc, manganese are also contained in substantial amount in *C. forda* larva. Zinc is important because its deficiency can lead to growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, impaired appetite, increase susceptibility ti infection mediated via defects in the immune system (FAO/WHO, 2001).

5.1.5.3 Vitamin Content of *C. forda* and vegetable soups

Generally, insects have been reported to contain a varying degree of both water-soluble and fat-soluble. *C. forda* was found to contain a tangible amount of B vitamins, vitamin C and E. In *C. forda*, vitamin C was the most abundant. Vitamin C helps in maintaining blood vessel flexibility and improves circulation in the arteries (Alamu *et al.,* 2013). The Addition of *Egusi* and *C. forda* led to a significant increase in the vitamin content of the vegetable soups C, D and E.

5.1.5.4 Antinutrient level of *C. forda* and vegetable soups

The absence of oxalates, tannins and saponins and negligible phytate content in *C. forda* from (Table 4.30) and is believed to be due to food conversion of the leaves consumed by the larvae during the process of entering the soil before being harvested as its names implies in Yoruba Language (*Kanni – wole* meaning the larva has entered the soil) Osasona and Olaofe, 2010. The phytates, the oxalate, tannin and saponin content of the *C. forda* in this study was much lower than those reported by Alamu *et al.,* 2013, Omotosho and Adesola (2018) Anti-nutrients are generally known to reduce the bioavailability of nutrient in the body. High phytate level in human nutrition decreases the availability of some minerals such as Calcium, magnesium, and

Iron by the formation of insoluble materials (Anuonye *et al.,* 2012) while tannin reduce bioavailability when bound to protein to induce a decrease in solubility and functionality of the protein

5.1.5.5 Amino acid profile of *C. forda* larva and vegetable soups

C. forda larva contained a wide array of both essential and non-essential amino acid as shown in table 4.31. the quality of a food protein depends on its amino acid composition. *C forda* was rich in essential amino acid, it contained all the nine (9) essential amino acid with Lysine been the most predominant amino acid in *C. forda* follow by lysine, glutamic acid, threonine, aspartic acid, leucine, glycine, isoleucine, tyrosine and phenylalanine. The amino acid isan important component for healing and protein synthesis processes. Glycine together with other amino acids such as alanine, arginine and phenylalanine form a polypeptide that promotes growth and tissue healing (Witte *et al.,*2002; Adeoti *et al.,* 2013) The high content of essential amino acid in *Cirina forda* is very beneficial as this implied that protein is of high biological value. The addition of *C. forda* larva to enrich the vegetable soups C and E significantly increased their protein content ($p<0.05$)

5.1.5.6 Fatty acid profile of *C. forda* larva and vegetable soups

The larva of *C. forda* containsa high amount of poly-unsaturated fatty acid (PUFA), (33.05% linolenic acid and 7.07% linoleic acid). The ratio of the poly-unsaturated to saturated fatty acid (P/S) has been used widely to indicate the cholesterol-lowering potential of food (Akinowo and Ketiku, 2000) They reported that a P/S ratio of 0.2 has been associated with cholesterol level with a high risk of coronary heart disorders. The P/S ratio of *C. forda* larva in this study is 0.8, and a ratio as high as 0.8 has been reported to be associated with desirable levels of cholesterol and reduction of coronary heart disease (Akinawo and Ketiku, 2000). Thus regular consumption

of *C. forda* could help in the intake of healthy that can prevent the onset of cardiovascular diseases

5.1.6Microbiological assessment of *Cirina forda*larva when stored for 90 and 180 days

The term *shelf life* is the length of time that food, drink, medicine, chemicals, and many other perishable items are given before they are considered unsuitable for sale, use, or consumption. Shelf life is also the recommendation of time that products can be stored, during which the defined quality of a specified proportion of the product remains acceptable under expected or specified conditions of distribution, storage and display (Anon, 2003).Micro-organisms play a substantial role in determining the storage span of food products as they are typically accountable for the decomposition of many foods.

The refrigerated samples in this study had less heterotrophic count than the samples stored at ambient temperature for 90 days. However, after 180 days of storage, the samples stored at ambient temperature had completely gone bad and had turned into powder. Microbial analysis showed that the total heterotrophic counts for the microflora from the refrigerated samples were within acceptable limit even after storage for 180 days. However, dried larva should be exposed to more heat before consumption as the microflora present could be easily destroyed by cooking at 100°C.

The Proximate, mineral and vitamin contents of the refrigerated samples were higher than those stored at ambient temperature at 90 days.The refrigerated sample after 180 days of storage had a slight reduction in their nutrient composition when compared to storage at 90 days and the first day of the study. Nutrient composition decreases with storage time, Banjo, *et al.,* (2013).

Summary of Discussions

Based on the result obtained from inferential statistics of different components of *Cirina forda.* It could be observed in Table 4.10 shows that proximate analysis of minerals, vitamins and anti-nutritional content are the same in the samples obtained from different locations. This finding is in alliance with Banjo *et al.,* (2006) and Osasona and Olaofe (2010) who reported that there is no significant location different in vitamins and anti-nutritional content across the different locations. Also, the finding from the comparison of amino-acid composition of various samples of *Cirina forda* larva taken from Nigeria, Ghana and Burkina Faso as used in the study revealed that even the amino-acid composition of the insect show no variation in their constitution. This is contrary to the theoretical expectation that location does mutate the genetic constitution of both plant and animal species. However, the result is in alliance with the findings of Zuraini *et al.,* (2006) who affirmed the abundance of amino-acid in the *Cirina forda* larva and observed no significant difference in the content of the amino acids across different samples from the various location as shown in Table 4.12.

Moreover, the finding in Table 4.14 revealed the result of the comparison of the fatty acid composition of *Cirina forda* larva in the selected locations. It was found that *Cirina forda* larva does not significantly vary in fatty acid composition based on the location where the sample was drawn. The result corroborates previous findings ofAdepoju and Daboh (2020). In all indications, an inference could be made that *Cirina forda* larva composition does not vary in constitutions such as vitamin, fatty acid, amino-acid and minerals component with geographical locations, although the climatic factors of the sample locations in the study were not established. Therefore, it could be said that there is no variation in the nutritional value of *Cirina forda* larva obtained from a different location.

5.2 Conclusion

This study confirmed that *Cirina forda* larvae are most popular among the edible insects relished in the study area as most of the respondents were aware of its existence and often consume it. It is usually roasted, boiled, fried, or powder forms but majorly as condiments in the soup. The major reason for its consumption by the respondent include among others its flavour and taste, food habits/custom, nutritional benefits and economic preferences. However scarcity in the market, civilization, marriage and possible adulteration with poisonous are the limitations to the consumption of the larvae.It was established in the study that religion or traditional belief or myth do not affect its consumption in the study location.The majority of the respondents are not aware of the nutritional benefits of the insect larvae hence there is a need to create a wide public enlightenment programme on the nutrient and health-importance to improve its consumption alongside some other edile popular insect in the community especially among the children and young adults.

Cirina forda was very rich in protein, high in fat and essential minerals and very low in antinutrient and compare favourably among other popular edible insects in the study area. The amino acid composition of the larva showed that it contained all the essential amino acids needed for human growth in good proportion, hence the protein content can be considered as a complete protein of high biological value, more so, it is of animal origin. *Cirina forda* has a huge store of lysine which can help substitute as a complement protein in the diets of mostly predominantly grain-dependent communities. The fat content of the insect larva contained a higher amount of unsaturated fatty acid compared with thesaturated acid showing that it can serve as a good source of healthy fat that is fit for human consumption for the promotion of good health. The insect larva and the enriched vegetable soups contained a negligible level of anti-nutrients which cannot pose any threat to nutrient bioavailability from the larva and the vegetable soup, hence it is believed that the consumption of the insect larva either as snacks or as a condiment in vegetable soups or sauces is very safe and will promote quality nutrient intake by the consumers.

The soups enriched with*Cirina forda* weremore acceptable than the plain vegetable or vegetable with *Egusi* soup. The *Cirina forda*-larva enriched *Egusi* soup was the most acceptablein terms of colour, taste, aroma and overall acceptability. The inclusion of *C.forda* larva in vegetable soups

improve both its palatability and nutrient content therefore, its inclusion in soups and sauces should be encouraged.

The serum analysis of the nutrient bioavailability shows that protein, minerals, and vitamin A were bioavailable in rats' bodies. This signifies that *Cirina forda* larva consumption supports growth in animals.

Under the conditions described for the administration of *Cirina forda* larva powder in this study, there are no toxicological effects on body weight change, feed intake, phytochemical screening, urinalysis, haematological or serum biochemical analysis, organ weight, or histopathological analysis,

Microbial analysis to examine the shelf-life of the *Cirina forda* larva samples showed that the total heterotrophic counts for the microflora obtained are still within the acceptable limit even after storage for 180 days, as the microflora present could be easily destroyed by cooking at 100°C. The result showed it keeps best under refrigeration temperature than at the normal ambient condition.

5.3 Recommendations

Based on the findings of the study, it could be recommended that:

1. Pregnant women and children who are deficit of essential amino-acid most importantly lysine could consume *Cirina forda* or use it as a supplement as a condiment in soups such as okro, vegetable and stews.
2. *Cirina forda* could be consumed among old age people to reduce the risk of heart diseases (heart and blood vessel harden) or attack, lower blood pressure and cholesterol because it is high in linolenic acid.
3. Popularizing the consumption of this insect should be encouraged as a means of increasing dietary diversity of the population of people where the insect is readily available, this will help to promote the intake of quality protein and essential minerals among the populace of the host community and assist in combating protein and micronutrient malnutrition thereby improving the general health of the people also

itconsumption could serve as means of conserving host tree from going into extinction due to human activities.

4. If it gainsa high level of acceptance or publicity, *Cirina forda* could be made as a complementary food for growing up children or incorporating it into insect-based flour for biscuit, bread, cookies chips and paster.
5. Due to the nutritional benefits of *Cirina forda*, stakeholders such as nutritionists, botanists, government and individual members of communities should manage and preserve the host plants to prevent their extinction due to deforestation and other ecological threats.

5.4 Contribution to Knowledge

The findings from this study widen the scope and conceptualization of the nutritionist and consumers of *Cirina forda*concerning its accessibility, palatability and shelf-life. The presence of *Cirina forda* in the three West African countries shows that *Cirina forda* is known and has wider coverage in term of geographical distribution, this underlines the reason why is widely consumed among the populace in the study area. The study also documented the fact that *Cirina forda* possesses quality high protein having all essential amino acids present in it which are consumable and could supplement human nutritional need most especially, children, pregnant and lactating women. Protein in *Cirina forda* is bioavailable and support growth in rats and safe for human consumption. The low MUFA content of insects, for example in *Cirina forda* as confirmed in the study has led some researchers – such as those involved in the FAO report – to suggest that entomophagy may be an effective way to combat obesity and its related diseases.Reports had showed that regular consumption or the addition of edible insects in diets help in losing weight.Besides, it was found at the course of the study that *Cirina forda* is enriched with quality vitamins such as vitamins; C, B_{12} and E and minerals such as Potassium is most abundant and favourably high in iron and zinc. The study also reveals that *Cirina forda* can be stored under favourable condition for more than six months, which could be a favourable factor in promoting the edible insect'scontribution to food and nutrient security.Conclusively, the study was able to establish the fact that geographical location does not affect the nutritional contents of *Cirina forda*larva.

5.5.2 Consideration for Further Studies

Consideration for further studies after this work will be;

- To determine the effect of feeding rats with higher quantities of *Cirina forda* powder used in this study and a much longer period on the rats liver organ weights in relation to the level of Alkaline phosphatase, ALP in the rats' livers to ascertain if the consumption of this edible could be hepatoxic. Although, Akinnawo and Ketiku, (2002) revealed that the processed larva of *Cirina forda* is neither neurotoxic nor hepatoxic to rats.
- To evaluate the functional properties of the mixed-powder when blended with the wheat flour for the production of bread, pasta and biscuit.
- To determine if heavy metals such as lead, mercury, cyanide compounds etc. are present in *Cirina forda* larva

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APPENDIX 1: QUESTIONAIRE

CONSUMPTION SURVEY OF EDIBLE GIANT SILKWORM, *Cirina forda*(WESTWOOD) LARVAE-MONIMONI IN SOUTHWESTERN NIGERIA

A . SOCIO-DEMOGRAPHIC SECTION

Name: ……Age:……

Sex…… Occupation: …….. Tribe: ……..

Religion: ……

Level of Education: ……. Family Size:……

Family Income:…….

Ward: …… LGA: ……

State: …….

B. CONSUMPTION PATTERN AND FREQUENCY OF CONSUMPTION OF *CIRINA FORDA* (MONIMONI/KANNI)

1. Do you know *Cirina forda* (Monimoni/Kanni)? Yes ( ) No ( )

2. What name is it called in your local language?

.....

3. Have you consumed *Cirina forda* (Monimoni/Kanni) before? Yes ( ) No ( )

4. When do you start consuming *Cirina forda* (Monimoni/Kanni)? Since childhood ( ), Adolescence ( ), Adulthood ( )

5. Do you consume *Cirina forda* (MoniMoni/Kanni) often? Yes ( ) No ( )

6. If yes, how often? Daily ( ), Weekly ( ), Monthly ( ), Seldomly ( )

7. Do other members of your household consume *Cirina forda* (MoniMoni/Kanni)? Yes ( ) No ( )

8. If yes, how often do you consume *Cirina forda* (MoniMoni/Kanni)? (Regularly/Seldomly)

9. What socio-economic class of people do you know that consume *Cirina forda* (MoniMoni/Kanni)?

Rich ( ) Average ( ) Poor ( )

10. Which group of people do you know that consume *Cirina forda* (MoniMoni/Kanni)?

Children ( ) Adolescents ( ) Adult ( ) Everybody ( )

11. In what form do you consume *Cirina forda* (MoniMoni/Kanni)?

Roasted/dried ( ) Boiled ( ) Powdered ( )

12. How do you prefer its consumption? Snack ( ), Spice in food ( ), Condiment in soup ( )

13. If you prefer it Q.12 as a condiment in soup, what soups do you like it with 1. ……., 2. ……., 3. ……

14. Is there a difference between its consumption rate in the past and present? Yes ( ), No ( )

15. If yes, what is the difference?

……

16. Can you mention other edible insects' people consume in your locality here?

1. ……

2. ……

3. ……

4. ……

17. Which one do you like to consume most?

……..

C. AVAILABILITY AND ACCESSIBILITY

18. How popular and/or acceptable is *Cirina forda* (Monimoni/Kanni) in your locality here?

Very popular ( ) Popular ( ) Rarely known ( ) Not known at all ( )

19. Is *Cirina forda* (Monimoni/Kanni) available all year round? Yes ( ) No ( )

20. If No, what season of the year is it mostly available? Rainy ( ) Dry ( )

21. Can *Cirina forda* (Monimoni/Kanni) be stored/preserved? Yes ( ) No ( )

22. If yes, in what form is it stored/preserved? Dried ( ) Wet ( ) others ()

23. For how long can it be stored/preserved? 1-3 months ( ), 4-6 months ( ), 7-12months ( )

24. Is *Cirina forda* (MoniMoni/Kanni) available in the mark*et al.,*l year round? Yes ( ) No ( )

25. Is *Cirina forda* (MoniMoni/Kanni) affordable in the market for all? Yes ( ) No ( )

26. What is the average cost? Per 100g……. Mudu /rubber ……

D . CONSUMER AWARENESS AND BENEFITS OF CONSUMPTION TO HEALTH

27. Why do you consume *Cirina forda* (Monimoni/Kanni)? For Flavour and taste ( ), Food habit/Custom ( ), Nutritional/health benefits ( )

28. Please specify the nutritional benefit you know……..

……

29. What are the health benefits of *Cirina forda* (Monimoni/Kanni) to consumers?

1……

2……

3……

30. Do you know any health/toxic related issue(s) related to its consumption? Yes ( ), No ( )

31. If yes, what are they?

1……

2……

E. LIMITATION AND RESTRICTION TO CONSUMPTION

32. Does religious belief affect its consumption? Yes ( ) No ( )

33. If yes, in what ways?

.....,……

34. Is there any traditional belief/myths attached to its consumption? Yes ( ) No ( )

35.. If yes, what do people say about its consumption?

……..

36. What side effect(s) do you notice upon consumption of *Cirina forda*……

37. Does availability affect its consumption, Yes ( ) No ( )

38. If yes, in what ways

……,……,……

39. Does age affect its consumption? Yes ( ), No ( )

40. If yes, which age group consumes it? Children (2-12), Teenagers (13.19), Young (20-35), Adult (≥36)

41. Does cost affect it consumption? Yes ( ) No ( )

42. If yes, How? It is too expensive? Yes ( ) No ( ); Is the price cheap? Yes ( ) No ( )

43. What is the general opinion of people in your locality concerning *Cirina forda* (MoniMoni/Kanni)
consumption?……
……
……

44. Do you prefer it to meat, egg or fish? Yes ( ), No ( )

45. Will continue to consume *Cirina forda* ? Yes ( ), No ( ).

APPENDIX 2: Table for the Hippocratic Screen according to Malone *et al.,*

Date:		
Animal tested:	Sex:	Weight:
Drug used:	Vehicle:	Concentration:
Volume injected:	Administration route:	Dosage:

Identification mark: N°: Color:

Activity		Parameters	Time of injection																				
			Minutes			Hours				Days													
			0	15	30	1	2	4	8	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CENTRAL NERVOUS SYSTEM	STIMULATING	>Motility																					
		>Respiratory frequency																					
		Tail erection																					
		Exophthalmus																					
		Stereotype movements																					
		Paw licking																					
		Mouth scratching																					
		Tail biting																					
		Clonic convulsions																					
		Tonic convulsions																					
		Fine tremors																					
		Coarse tremors																					
	DEPRESSING	<Motility																					
		<Respiratory frequency																					
		Catatonia																					
		Palpebral ptosis																					
		Analgesia																					
		Anaesthesia																					
		Loss corneal reflex																					
		Ataxia																					
		Dyspnea																					
		Alienation of environment																					
		Back tonus																					
		Loss of … (paw)																					
		Exophthalmia																					
		Paralysis																					
		Sedation																					
OTHER EFFECTS	EAR	Pallor																					
		Cyanosis																					
		Hyperemia																					
	Urination	Increased																					
		Decreased																					
		Color																					
		Diarrhea																					
		Contortion																					
		Reaction of flight																					
		Passivity																					
		Aggressiveness																					
		Grunts																					
		Drooling																					
		Fasciculation																					

	Mydriasis																				
	Tail erection																				
	Tail tremor																				
	Pupil diameter																				

Heart	Arrhythmia	Stop systole	Diastole
Intestines	Motility	No motility	Hypermotility
Liver			
Lungs			
Blood	Pre-coagulated	Coagulated	Not coagulated
Gallbladder	Extended	Normal	Full
Other observations			

Appendix 3: Instrument Setting for Atomic Absorption Spectrophotometry

MODEL: BUCK 211VGP, MADE BY BUCK SCIENTIFIC.

H = ELEMENT, A = WAVELENGTH (nm), B = SLIT WIDTH (nm), C = WORKING RANGE (μg/ml), D = SENSITIVITY (μg/ml), E = LAMP CURRENT, F = FLAME TYPE.

Table 3.1: Instrument setting for AAS

H	A	B	C	D	E	F
Ca	422.7	0.5	1-4	0.02	10.0	N2O-C2H2
Ca	422.7	0.5	1-10	0.09	10.0	Air-C2H2
Cu	324.7	0.5	1-20	0.1	15.0	Air-C2H2
Cu	217.9	0.2	7.5-30	0.2	15.0	Air-C2H2
Fe	248.3	0.2	2-20	0.1	30.0	Air-C2H2
Fe	372.0	0.2	20-80	0.5	30.0	Air-C2H2
K	766.5	0.5	1-10	0.01	6.0	Air-C2H2
Mg	285.2	0.5	0.1-2	0.01	6.0	Air-C2H2
Mg	202.6	1.0	5-20	0.1	6.0	Air-C2H2
Mn	279.5	0.2	1-10	0.06	5.0	Air-C2H2
Mn	403.1	0.2	7-27	0.2	5.0	Air-C2H2
Na	589.0	0.2	0.03-1	0.02	8.0	Air-C2H2
Zn	213.9	0.5	0.5-5	0.03	15.0	Air-C2H2

Appendix 4: Table of Amino acid absorbance

AMINO ACID	WAVELENGTH	COLOUR
ASPARAGINE	505nm	LIGHT BLUE
ALANINE	620nm	BLUE
ASPARTIC ACID	465nm	PURPLE
CYSTEINE	600nm	BLUE
GLUTAMIC ACID	560nm	PURPLE
GLYCINE	525nm	PURPLE
HISTIDINE	460nm	PURPLE
ISOLEUCINE	580nm	LIGHT PURPLE
LEUCINE	570nm	PURPLE
LYSINE	450nm	ORANGE YELLOW
METHIONINE	525nm	GREENISH YELLOW
ORITHINE	590nm	PURPLE
PHENYLALANINE	545nm	YELLOW
PROLINE	470nm	YELLOW
PYRROLYSINE	455nm	YELLOWISH BLUE
SERINE	485nm	BLUE
THREONINE	615nm	BLUISH GREEN
TYROSINE	530nm	GREENISH BLUE
TRYTOPHAN	565nm	YELLOWISH BLUE
VALINE	490nm	GREENISH BLUE

Appendix 5:Plate of Animal experiment setup at the Laboratory

Appendix 6: Plate of The Researcher carrying out Urinalysis test

Appendix 7: Slides of Vegetable Soup Samples for the sensory evaluation test

Sample B: Vegetable Soup

Sample C: Vegetable Soup enriched with *Cirinaforda*

Sample D: *Egusi* (melon) soup (control)

Sample E: *Egusi* enriched with *Cirina forda*

Appendix 8: The study protocol was ratified by the Joint Institutional Review Board of the University of Ibadan and University College Hospital, Ibadan (UI/EC/16/0276).

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)
College of Medicine, University of Ibadan, Ibadan, Nigeria.

Director: **Prof. Catherine O. Falade,** *MBBS (Ib), M.Sc., FMCP, FWACP*
Tel: 0803 326 4593, 0802 360 9151
e-mail: cfalade@comui.edu.ng lillyfunke@yahoo.com

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Nutrient Composition and Dietary Assessment of *Cirina forda* (Westwood) Enriched Delicacies/Vegetable Soups to Nutrient intake of Consumers

UI/UCH Ethics Committee assigned number: UI/EC/16/0276

Name of Principal Investigator: **Oladele O. Daboh**
Address of Principal Investigator: Department of Human Nutrition
College of Medicine
University of Ibadan, Ibadan

Date of receipt of valid application: 19/08/2016

Date of meeting when final determination on ethical approval was made: **27/04/2017**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from **27/04/2017 to 26/04/2018.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

Professor Catherine O. Falade
Director, IAMRAT
Chairperson, UI/UCH Ethics Committee
E-mail: uiuchec@gmail.com

Appendix 9: : TheUniversity of Ibadan Animal Care and Use Research Ethics Committee approved the animal model experimentations (UI-ACUREC/19/0034).

UNIVERSITY OF IBADAN
ANIMAL CARE AND USE RESEARCH ETHICS COMMITTEE
(UI-ACUREC)
E.mail: animaluseresearch@gmail.com/ animaluseresearch@yahoo.com
08176917269

Our Ref: ..... Date: 21-5-2019

Assigned Number: UI-ACUREC/19/0034

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Nutrient Composition, Bioavailability and Consumer Acceptability of *Cirina forda* (Westwood) Enriched Vegetable Soups

Name and Address of Principal Investigator: Daboh Oladele Olatunji
Department of Human Nutrition
Faculty of Public Health
University of Ibadan, Ibadan

Date of receipt of valid application: 5/3/2019

Date of meeting when final determination on ethical approval was made: 15/5/2019

This is to inform you that the research described in the submitted protocol, have been received and *given full approval by the* UI-ACUREC. Please, note that the Committee has approved only the animal component of the study. This approval dates from **15/5/2019 to 14/5/2020.** If there is delay in starting the research, please inform UI-ACUREC so that the dates of approval can be adjusted accordingly.

Note that no activity related to this research may be conducted outside of these dates. It is expected that you submit your annual report as well as an annual request for the project renewal to the UI-ACUREC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the code including ensuring that all adverse events are reported promptly to the UI-ACUREC. No changes are permitted in the research without prior approval by the UI-ACUREC except in circumstances outlined in the code.

You are to note that UI-ACUREC reserves the right to monitor and conduct compliance visit to your research site without previous notification.

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Prof. S. I. B. Cadmus
Chairman, UI-ACUREC

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Appendix 10: Variation in Proximate Analysis, Mineral Composition, Vitamins, and Anti-Nutritional content of the *Cirina forda* Samples across locations

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	78.344	2	39.172		
Within Groups	478762.081	96	4987.105	.008	.992
Total	478840.425	98			

One-Way analysis of variance (ANOVA) was used to examine variation in minerals composition, vitamin and anti-nutritional content of *Cirina forda*samples obtained from different locations (Ghana, Nigeria and Burkina Faso). The result revealed that no significant difference was found ($F_{(2,96)}$=0.008, p>0.05). This implies that the mean minerals, vitamins and anti-nutritional composition of *Cirina forda*did not vary across locations

Appendix 11: Difference in Amino Acid composition of the samples across Location

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.082	2	.041		
Within Groups	1264.243	57	22.180	.002	.998
Total	1264.325	59			

Amino-Acid composition of different samples of *Cirina forda* obtained from Ghana, Nigeria and Burkina Faso was compared using one-way analysis of variance (ANOVA) as shown in Table above. The result revealed that no significant difference was found ($F_{(2,57)}$= .001, p>0.05), which is an indication that the fatty acid composition of *Cirina forda* is the function of location.

Appendix 12: Difference in Fatty Acids Composition of the Sample across locations

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.158	2	.079	.001	.999
Within Groups	4951.382	45	110.031		
Total	4951.541	47			

The table above presents the result of the analysis on the difference in Fatty Acids composition of *Cirina forda samples* based on location (Ghana, Nigeria and Burkina Faso). The result shows that there is no significant mean differencebetween the three samples ($F_{(2,45)}$= .001, p>0.05). Thus, an inference could be made that the Fatty Acid Composition of the *Cirina forda* is constant across the different locations.

Appendix 13: Difference in Sensory Evaluation of the various *Cirina forda* Soups Across Groups

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2048.320	3	512.080	.053	.995
Within Groups	1386740.671	143	9697.487		
Total	1388788.991	146			

Appendix 13 presents the result of the analysis on the difference in sensory evaluation of four different types of soups prepared using *Cirina forda.* The mean sensory evaluation was compared using a One-Way analysis of variance and the result revealed that there is no statistically significant different ($F_{(4,143)}$= .001, p>0.05).