EFFECTS OF CASSAVA FLAKES DIET FORTIFIED WITH PROTEIN FROM OPTIMIZED *CIRINA FORDA* ON GROWTH, HISTOPATHOLOGY AND HAEMATOLOGICAL PARAMETERS IN ALBINO RATS

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ABSTRACT

Hunger and malnutrition continue to pose serious problems for many people in most developing countries including Nigeria. Insects have been reported to be rich in protein and other micronutrients but their consumption is limited by acceptability and seasonal availability. This study was carried out to determine the effects of cassava flakes diet fortified with protein from optimized *Cirina forda* on growth, histopathology and hematological parameters in albino rats. Experimental diets were formulated using soyabean, growth optimized and non-optimized *Cirina forda* as sources of protein, and cassava flakes as the basal diet. Proximate, anti-nutrient and mineral compositions were carried out using AOAC method while amino acid analysis was carried out on PTH Amino Acid Analyzer (Model 120A). Thirty (30) albino rats with average weight of 107.57 were divided into six (6) groups of 5 rats each and fed with various formulated diets and water ad libitium for 21 days. Group A (control) were fed with normal rat chow, while Groups B and C were fed with 10% and 20% soyabean protein (standard protein diets) respectively. Group D was fed 10% optimized *Cirina forda* protein, while Groups E and F were fed 10% non-optimized *Cirina forda* protein and 20% non-optimized *Cirina forda* protein respectively. Their weekly body weight and daily feed intake were measured. After the experimental period, the rats were sacrificed, blood samples collected and the liver, kidney and intestine excised for histopathological analyses. The hematological analyses were determined using auto-haematological analyser (Mindray BC-5300). Liver and kidney function tests were carried out using Spectrum, Teco and Agappe diagnostic assay kits. Histopathological analysis of the organs was carried out using Haematoxylin and Eosin (H&E) staining microscopic technique. Protein values for optimized *Cirina forda* (55.345 ±0.345) was significantly higher ($P<0.05$) than that of the non-optimised *Cirina forda* (51.335 ± 0.425). The weight gain in animals placed on 20% soyabean protein was significantly higher ($P\leq0.05$) than in any other group, while the weight gain in animals fed with the non-optimised, optimized *Cirina forda* and rat chow were similar. This same trend is true for the organ/body weight ratios. The heamatological parameters, kidney function and liver function parameters though varied in all the groups were all within normal range. There was no abnormality in the histo-architecture of all the groups studied. The findings in this work showed that feeding of rats with cassava flakes supplemented with *Cirina forda* gave similar results with that of the 10% soybean diet and the control. The use of *Cirina forda* in diets may therefore find relevance in solving protein energy malnutrition (PEM) due to its high essential amino acids.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Malnutrition is a health challenge predominant in Nigeria especially the protein energy malnutrition which results from protein deficiency (WHO, 2014). This is as a result of total or overdependence on conventional food sources which are limited in protein supply. Protein-energy malnutrition (lack of protein-energy giving foods) is a range of mild, moderate to pathological conditions arising from deficiency in protein and energy (WHO & UNICEF, 2013).

According to World Food Program (McGuire, 2015) about 805 million people are unable to maintain a healthy living. Staple foods consumed in Africa are mainly tubers and cereals grains (Filli *et al*., 2014). These foods are composed of 70-80 % carbohydrate and high consumption rate of these staple foods could lead to malnutrition especially protein. This has posed significant health challenge for developing countries. Malnutrition in the world has severe consequences on human and has been reported to be the most important risk factor for illness and death from diseases (Muller *et al*., 2005; McGuire, 2015). The underlying causes of malnutrition have been attributed to drought and conflict, rapid population growth, poverty, ignorance and fall in food production. For instance, deforestation, a largely unsustainable agricultural process has resulted in soil erosion and even destruction of rice field (a major staple) (WHO & UNICEF, 2013). The fragility of the soil has left very few farmers with enough land to cultivate. Given that agriculture accounts for more than one-fourth of the country's gross domestic product (GDP) and 80 % of

employment, this left a large percentage of the population vulnerable to food insecurity (FAO, 2013).

Protein energy malnutrition (PEM) is a form of malnutrition arising from lack of dietary protein and /or energy (calories) in varying proportions. PEM is common and it is connection with chronic diseases and increased morbidity and mortality. It affects both children and adults and accounts for 6 million death annually (FAO, 2013). Apart from protein energy malnutrition, there is also micronutrient malnutrition which results from deficiency of specific micronutrient essential for a healthy life. A major concern for developing countries is protein and iron intake as their deficiencies are the world's most common nutritive disorder (WHO, 2014). However, consumption of underutilized food materials such as insects could be relevant in alleviating protein energy malnutrition. Many of the insects safe for consumption contain abundant levels of iron that often exceed other commonly eaten animals.

Edible insects such as palm weevils (*Rhynchophorusphoenicis*) or mopane caterpillars (*Imbrasiabelina*), both species found in Africa, can provide 12 to 31 mg of iron per 100 g of weight, respectively (Banjo *et al*., 2011). Chicken and beef, on the other hand, provide only 1.2 and 3 mg of iron, respectively (Sirimungkararat *et al*., 2010). Zinc another mineral important for growth and development, can be generally found in most insects. It has been reported that palm weevil larvae contain 26.5 mg protein per 100 g of sample as well as other minerals important for growth and development. Protein and mineral nutrients can generally be found in most insects (Van Huis *et al.,* 2013).

Insects are invertebrate animals belonging to the class insecta.They constitute the most abundant multicellular organisms on planet Earth and are more than 70 % of all

species(Van Huis *et al*., 2013). Their actual number is believed to range from 2.5 and 10 million, but only one million species have been classified and named (Yang *et al.,* 2012). Within the insects orders; Coleoptera, Hymenoptera, Orthoptera, and Lepidoptera (caterpillars) represent 80 % of edible species.Recently, insects have received wide attention as a potential alternate major source of protein (Makkar*et al*., 2014), due to their nutritional content comparable to conventional livestock and fish.

The crude protein content of most insects ranges from 40 to 75 % on dry weight basis, with beneficial amino acid profile, and a variable fat content. Insects are also valuable sources of minerals and vitamins (Finke, 2013). They have a high biodiversity with higher feed conversion efficiency than beef (Yang *et al*., 2012). Insects constitute quality food for humans and emit low levels of greenhouse gasses (Van Huis *et al*., 2013). They have great potentials as resource for relieving global food insecurity and malnutrition. Palm weevils have been traditional African diets for centuries in South Africa (Banjo *et al*., 2006). Studies have indicated high protein levels in grasshoppers fed with bran (Ademolu *et al*., 2010). Edible insects can be incorporated into other foods, such as south-Africa ground giant water bugs which are essential in makingchilli paste and fish source (kiatbenjakul *et al*., 2015).

Cirina forda (moth caterpillar), a lepidopterous insect, is a pest of the shea butter tree and feeds on leaves of *eurytropheum suavelens* (Lisingo *et al*., 2010). *Cirina forda* has been reported to contain about 63 % protein and 14 % lipid. Also, *Cirina forda* was reported to be a rich source of calorie and minerals (Osasona & Olaofe, 2010). It is less expensive and could be easily incorporated into diets to substitute conventional protein sources in the feeding of animals such as Catfish and Rats (kiatbenjakul *et al*., 2015).The larva of this

insect resembles silk worm caterpillars but they do not spin cocoons. Instead, they dig into the soil at the base of the host tree to pupate. They can be processed into the dried form which is widely marketed and consumed as an essential ingredient in vegeTable soups (Van Huis *et al*., 2013). The dried larva can also be used as human diets or animal feed formulation, and could be taken with foods rich in carbohydrate (Omotosho, 2006). *Cirina forda* is reported to be one of the most important and widely eaten insects in some parts of Nigeria, especially the southern Nigeria (Dunkel, 2015).The caterpillars of this butterfly are harvested in the rain season (June to August) and marketed throughout the year in southwestern Bukina Faso, Togo and Moba (Filli, 2014).

Entomophagy has long been accepted in developing countries. For instance, countries such as India and Lao People's Democratic Republic have identified 24 and 21 edible insect species, respectively. Also, Africa has been reported to have some 246 species of edible insects. Countries within this continent that exhibit more severe rates of chronic malnutrition, such as Madagascar or Zambia, possess 22 to 33 species (Ramos-Elorduy *et al*., 2011). These insects are consumed for their comparable levels of energy and nutrients, although the levels may vary depending on species type, metamorphic stage, habitat, and diet. A study conducted in Thailand demonstrated that 100 g of insects (fresh weight) had comparable, if not more, calories than that of equal weights of commonly eaten livestock (Sirimungkararat *et al*., 2010).

Edible insects are also considered to be a substantial source of macronutrients especially protein. Certain insects found in China were reported with higher protein contents than those in most plants and commercial meat, fowl, and eggs (Xiaoming *et al*., 2010). In the study, 11 orders of insects were analyzed with results of protein contents ranging from a

low 13 % to a high 77 % (dry weight basis). (Raksakantong *et al*., 2010) also reported a range of 37–54 % protein content in eight insects found in Thailand. In spite of these variations in protein content, edible insects are known to be an exceptionally good source of protein. Macronutrient is often difficult to obtain in developing countries, hence it is lacking from daily diets of most people in this countries. Edible insects which are relatively more available than other meats had been demonstrated to be solution in alleviating protein energy malnutrition and hence reducing global food insecurity.

Cassava is a staple food for more than half of the world's population including Nigeria and most developing countries (FAO, 2013). It is the third source of food carbohydrate after rice and maize (Yang *et al.,* 2012) and is widely grown on marginal soils with Nigeria being the largest producer (FAO 2013). However, Cassava being a staple food is a carbohydrate based food and not a major source of protein. Food fortification could serve as a means to improve food nutrients thereby decreasing the effects of nutrient deficiency in man (FAO, 2013). Hence to overcome protein energy malnutrition, there is need to include protein rich foods materials into staple foods for healthy living. This study was therefore undertaken to determine the effects of Optimised *Cirina forda* and non-optimised *Cirina forda* protein fortified cassava flakes diets on growth performance, hematological and histopathological parameters of albino rats.

1.2 Statement of the Research Problem

Malnutrition is a long lasting problem especially in women and children. This has resulted in extremely high rate of mortality among citizens in Nigeria (Nsude & Nwanchor, 2017). An estimated number of two million children in Nigeria suffer from acute malnutrition (UNICEF, 2011). Staple foods usually consumed in Nigeria are deficient in protein content

and are majorly carbohydrate based foods. This has resulted to protein-energy malnutrition problems and on a large scale among the internally displaced persons (IDPs) (McGuire, 2015). The costs of obtaining protein from conventional sources of proteins are very expensive that most households cannot afford them, hence limiting supplies of these proteins to a few rich individuals who could afford them (Jacob *et al*., 2013). Although insects are very good and cheap sources of proteins, but the consumer acceptance of thesealternative sources of proteins in developing countries, Nigeria inclusive, are very poor. This poor acceptance of insects as alternative proteins sources for alleviating food protein-energy malnutrition further exacerbates the problem of protein-energy malnutrition. Also, the availability of protein rich insects such as *Cirina forda* is seasonal in nature which contributes to shortage in supply in places where they are accepted. The fact that these insects have short edible phase of growth further complicates supply problem because of limited availability. This further compounds the problem of protein-energy malnutrition.

1.3 Justification for the Study

Protein Energy Malnutrition (PEM) can be overcome through incorporation of foods rich in protein and minerals such as the underutilized insects (*Cirina forda*) into the commonly consumed protein deficient foods. *Cirina forda* are known to be very rich in protein and minerals making them suiTable for supplementation of conventional staple foods for human and animal consumptions.

Insects are cheap, easy to raise, quite common and easy to come by as compared with other conventional protein sources such as livestock. They have been found to have a very high feed conversion rate. Despite insects are high-protein, mostly edible food sources with high rate of feed conversion, they are underutilized. The paucity of data on the usability of

insects has been the major reason for their poor acceptability as food despite the numerous reports on their constituents. Insects are rich sources of proteins, calorie, vitamins, minerals and micronutrients. They are known to contain abundant levels of nutrient that often exceed other commonly eaten livestock and are safe for consumption (Banjo *et al*., 2006).

Cirina forda is well known and common in most African countries (Nigeria, Ghana, Zimbabwe, Democratic Republic of the Congo and South Africa). Several works have been carried out as it concerns its nutritional, functional and the toxicological properties of the edible larva. In the same vein, cassava is the third source of staple food (carbohydrate) after maize and rice in most African countries (Yang *et al.,* 2012). It is widely grown on marginal soils with Nigeria being the largest producers (FAO, 2013).

The fortification of cassava flakes (Garri) with *Soybean*, optimised and non-optimised *Cirina forda* protein could be an effective way of delivering valuable nutrients found in the insect into the cassava flakes for human and animal consumption. The fact cassava flakes is readily available and in abundance justified its use as the basal diet and the high-protein content of soybean and *Cirina forda* (optimised and non-optimised *Cirina forda*) justified their use as protein supplements.

1.4 Aim and Objectives of the study

1.4.1 Aim

The aim of this study is to determine the effects of soybean, optimised *Cirina forda* and non-optimised *Cirina forda* protein fortifiedcassava flakes diet on growth performance, hematological and histopathological indices in albino rats.

1.4.2 Objectives of the study

The objectives of the study are;

i. Determine the nutrient composition and anti-nutrient constituents present in cassava flakes, soybean and *Cirina forda;*

ii. Optimised *Cirina forda* for optimal nutrient availability for cassava flakes fortification;

iii. Determine the level of *Cirina forda* biomass to be incorporated into cassava flakes for nutrient optimization and improvement;

iv. Evaluate the performance of soybean, Optimised *Cirina forda* and non-optimised *Cirina forda* protein fortified cassava flakes diet on the weight and growth rate of albino rats;

v. Determine the effects of soybean, Optimised *Cirina forda* and non-optimised *Cirina forda* protein fortified cassava flakes diet on biochemical, hematological and histopathological parameters in the experimental rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Protein-Energy Malnutrition (PEM)

Protein-energy malnutrition (PEM) is serious problem worldwide and had been reported to be an important cause of secondary immune deficiency worldwide (McGuire, 2015). PEM may manifest as either marasmus, a wasting disease due to lack of protein and overall calories, or kwashiorkor, which presents with generalized oedema due to primary protein malnutrition. Kwashiorkor is caused by insufficient protein consumption, weaning of children to starchy foods and it is characterized by weight loss, arms, legs, decreased muscle mass, swollen abdomen ascites, increase of capillary permeability enlarged liver, fatty liver Peripheral oedema, decrease of oncotic pressure, anemia, lethargy hair and skin changes. While, Marasmus is typical of feature of energy deficiency disease, characterised by weight loss, “skin and bones” wasting, prominent of which is drastic ribs loss as well as adipose tissue growth retardation, chronic diarrhea, muscle atrophy and skin folding. The nutritional deficiencies in PEM are complex, frequently involving multiple micronutrient deficiencies as well as primary protein-calorie deficiencies (Muscaritoli *et al*., 2009).

The immunologic consequences of PEM are seen most markedly in the cellular or immune system response. Thymic atrophy has been well described in macronutrient deficiencies, with histologic findings of small lobules, loss in the differentiation ofcorticomedullary, decreased Hassell’s corpuscles, varying degrees of fibrosis, and reduced numbers of thymocytes. Wasting of the peripheral lymphoid organs may be seen as well, with lymphocyte depletion relatively confined to the T-cell regions of these tissues. Interestingly,

the abnormal thymic involution seen in malnutrition seems to be reversible if an adequate diet is reinstituted (Liu *et al*., 2001).

The complications of PEM includes; hypoglycemia, hypothermia, dehydration and shock, electrolyte imbalance and hypokalemia hyponatremia as well as infections (bacterial, viral and thrush) due to micronutrient deficiencies. However, PEM may be treated following progressive re-feeding, correction of water and electrolyte balance, treatment of infection or worm infestations, dietary support such as 3-4 g protein and 200 cal/ kg body weight/dayof vitamins and minerals (Ubesie *et al*., 2012).

2.1.1 Global prevalence of micronutrient malnutrition

Micronutrient malnutrition (MNM) is widespread problem, reported in the industrialized nations, but even more so in the developing regions of the world. It can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies. Micronutrient malnutrition has many adverse effects on human health, not all of which are clinically evident. Even moderate levels of deficiency (which cannot be detected by biochemical or clinical measurements) can have serious detrimental effects on human function (Dary & Hurrell, 2006).

The existence of MNM has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation. Worldwide, the three most common forms of MNM are iron, vitamin A and iodine deficiency. Together, these affect at least one third of the world's population, the majority of who are in developing countries. Of the four, iron deficiency is the most prevalent. It is estimated that just over 2 billion people are anaemic, just under 2 billion have inadequate iodine nutrition and 254 million preschool-aged children are

vitamin A deficient (WHO & UNICEF, 2013; Dary & Hurrell, 2006) MNM is a concern not just because such large numbers of people are affected, but also because MNM, being a risk factor for many diseases, can contribute to high rates of morbidity and even mortality. It has been estimated that micronutrient deficiencies account for about 7.3 % of the global burden of disease, with iron and vitamin A deficiency ranking among the 15 leading causes of the global disease burden (4). According to WHO mortality data, the health burden of MNM is huge with about 0.8 million deaths (1.5 % of the total) can be attributed to iron deficiency each year, and a similar number to vitamin A deficiency (McGuire, 2015).

In the poorer regions of the world, MNM is certain to exist wherever thereis undernutrition due to food shortages and is likely to be common where diets lack diversity. In Nigeria, particularly since the implementation of the sharia law in most parts of the northern Nigeria, considerable violence that rendered many killed and homeless degenerated into the coming into being of the boko haram. The insurgency carried by the sect in the northeast account for over 90 percent of the IDPs with less than 10 percent caused by natural disasters in Niger (Ibeanu, 2015) and rendering others refugees from foreign countries along the Nigeria border like Cameroun, Chad and Niger Republics. There are over 2,241,484 million IDPs in Nigeria and studies have shown that 13.33 % were due to communal clashes, 0.99 % by natural disaster and 85.68 % resulted from insurgency attacks (Nsude & Nwanchor, 2017).

Generally speaking, whereas wealthier population groups are able to augment dietary staples with micronutrient-rich foods (such as meat, fish, poultry, eggs, milk and dairy products) and have greater access to a variety of fruits and vegetables, poorer people tend to consume only small amounts of such foods, relying instead on more monotonous diets

based on cereals, roots and tubers (Gaur *et al.,* 2018). The micronutrient contents of cereals (especially after milling), roots and tubers are low, so these foods typically provide only a small proportion of the daily requirements for most vitamins and minerals. Fat intake among such groups is also often very low and given the role of fat in facilitating the absorption of a range of micronutrients across the gut wall, the low level of dietary fat puts such populations at further risk of MNM. Consequently, populations that consume little foods of animal source may suffer from a high prevalence of several micronutrient deficiencies (Table 2.1) simultaneously. In the wealthier countries, higher incomes, greater access to a wider variety of micronutrient-rich and fortified foods, and better health services, are all factors that contribute to the lowering of the risk and prevalence of MNM (Dary & Hurrell, 2006).

Table 2.1: Prevalence of the three major micronutrient deficiencies by WHO region

WHO region	Anaemia[a] (total population)		Insufficient iodine intake[b] (total population)		Vitamin A deficiency[c] (preschool children)	
	No. (millions)	% of total	No. (millions)	% of total	No. (millions)	% of total
Africa	244	46	260	43	53	49
Americas	141	19	75	10	16	20
South-East Asia	779	57	624	40	127	69
Europe	84	10	436	57	No data available	
Eastern Mediterranean	184	45	229	54	16	22
Western Pacific	598	38	365	24	42	27
Total	2030	37	1989	35	254	42

Source; (Dary & Hurrell, 2006)

2.1.2 Food fortification

Food fortification refers to the addition of micronutrients to processed foods. Fortification of foods has been identified as a means to decrease the effect of nutrient deficiency across the globe (FAO, 2013). This involves the addition of micronutrients to staple foods to prevent or ameliorate malnutrition in man and animals (Yang *et al.,* 2012). Food fortification according to the World Health Organization (WHO) and Food and Agricultural Organization of the United Nations is a way of increasing the essential micro nutrient content in food. Food fortification has been identified as a practice of reducing the occurrence of nutrient deficiencies in the world.

In many situations, this strategy can lead to relatively rapid improvements in the micronutrient status of a population, and at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks. Since the benefits are potentially large, food fortification can be a very cost-effective public health intervention. However, an obvious requirement is that the fortified food(s) needs to be consumed in adequate amounts by a large proportion of the target population. It is also necessary to have access to, and to use, fortificants that are well absorbed yet do not affect the sensory properties of foods. In most cases, it is preferable to use food vehicles that are centrally processed, and to have the support of the food industry (FAO, 2013).

Fortification of food with micronutrients is a valid technology for reducing micronutrient malnutrition as part of a food-based approach when and where existing food supplies and limited access fail to provide adequate levels of the respective nutrients in the diet. In such cases, food fortification reinforces and supports ongoing nutrition improvement programs

and should be regarded as part of a broader, integrated approach to prevent MNM, thereby complementing other approaches to improve micronutrient status.

2.2 Macronutrients and Micronutrients in Foods

2.2.1 Macronutrients

Macronutrients are needed in larger quantities (in gram range) in foods. They normally include water, carbohydrates, fat and protein. Macronutrients (except water) are also called energy-providing nutrients. Energy is measured in calories and is essential for the body to grow, repair and develop new tissues, conduct nerve impulses and regulate life process.

2.2.1 Carbohydrates

Carbohydrates are required for energy and provide body's main source of energy. They form the major part of stored food in the body for later use of energy and exist in three forms: sugar, starch and fiber. The brain works entirely on glucose alone. When in excess, it is stored in the liver as Glycogen. Carbohydrates are important for fat oxidation and can also be converted into protein (Byrne *et al.,* 2015).

2.2.2 Fats

Fats are important macronutrients which play major hormonal roles. Fats are used in making steroids and hormones and serve as solvents for hormones and fat soluble vitamins. Fats have the highest caloric content and provide the largest amount of energy when burnt. When measured by a calorimeter, fats provide about 9 calories per gram of fat, making them twice as energy-rich than protein and carbohydrates. Extra fat is stored in adipose tissue and is burnt when the body has run out of carbohydrates (Chatterjea & Shine, 2007).

2.2.3 Proteins

Proteins are very important macronutrients with roles in growth and development. They provide amino acids and make up most of the cell structure including the cell membrane. Proteins are the last of the macronutrients to be used after all carbohydrate and fat sources had been exhausted. In cases of extreme starvation, the muscles in the body, that are made upof proteins, are used to provide energy. This is called muscle wasting. Proteins provide 4calories of energy per gram (Marshall, 1986).

2.3 Micronutrients

These nutrients include minerals and vitamins. Unlike macronutrients, these are required in very minute amounts in the body. Together, they are extremely important for the normal functioning of the body. Their main function is to enable the many chemical reactions to occur in the body. Micronutrients do not function for the provision of energy (McGuire, 2015).

2.3.1 Vitamins

Vitamins are essential for normal metabolism, growth and development, and regulation of cell function. They work together with enzymes and other substances that are necessary for a healthy life. Vitamins are either fatsoluble or water-soluble. Fat soluble Vitamins can be stored in the fatty tissues in the body when in excess. Water soluble vitamins are excreted in urine when in excess and so need to be taken daily. Water soluble vitamins include Vitamin B and C. Green leafy vegetables are rich in Vitamin B, whereas Vitamin C is found abundantly in citrus fruits.Fat soluble vitamins are Vitamin A, D, E and K. Green leafy vegeTables, milk and dairy products and plant oils provide these vitamins (McDowell, 2008; Wardlaw*et al.,* 2004).

2.3.2 Minerals

Minerals are found in ionized form in the body. They are further classified into macrominerals and microminerals (or trace minerals). Macrominerals present in the body include calcium, potassium, iron, sodium and magnesium. Iron is a constituent of Hemoglobin which is present in blood. Macrominerals are needed in larger amounts compared to microminerals. Microminerals include copper, zinc, cobalt, chromium andfluoride. They are mostly co-factors, and are necessary for the function of enzymes in the body, but are needed onlyin minute quantities. Approximately 4 % of the body's mass consists of minerals (Raffety, 2011).

2.4 Insects, Roles and Benefits

2.4.1 Roles of insects in nature

Insects play a vital role in plant reproduction. An estimated 100,000 pollinator species have been identified and almost all of these (98 percent) are insects. Over 90 percent of the 250,000 flowering plant species depend on pollinators. This is also true for three-quarters of the 100 crop species that generate most of the world's food (Ingram *et al.,* 1996). Domesticated bees alone pollinate an estimated 15 percent of these species. Insects play an equally vital role in waste biodegradation. Beetle larvae, flies, ants and termites clean up dead plant matter, breaking down organic matter until it is fit to be consumed by fungi and bacteria. In this way, the minerals and nutrients of dead organisms become readily available in the soil for uptake by plants. Animal carcasses, for example, are consumed by fly maggots and beetle larvae. Dung beetles of which there are about 4,000 known species also play a significant role in decomposing manure (Slade *et al.,* 2016).

2.4.2 Roles of insects for humans

Apart from insects serving as sources of food, insects provide humans with a variety of other valuable products. Honey and silk are the most commonly known insect products. Bees provides about 1.2 million tonnes of commercial honey per year (Burlingame & Dernini, 2012), while silkworms produce more than 90,000 tonnes of silk (Yong-woo, 1999). Carmine, a red dye produced by scale insects (order Hemiptera), is used to colour foods, textiles and pharmaceuticals. Resilin, a rubber-like protein that enables insects to jump, has been used in medicine to repair arteries because of its elastic properties (Elvin *et al*., 2005). Other medical applications include maggot therapy and the use of bee products suchas honey, propolis, royal jelly and venom – in treating traumatic and infected wounds and burns (Van Huis, 2003). Insects have also inspired technology and engineering methods. The silk proteins of arthropods (example, spiders) are strong and elastic and have been used as biomaterials (Lewis, 1992). The unique structure of silk, its biocompatibility with living systems,its function as are tools for new materials engineering and its thermal stability are only a few of the features that make it a promising material for many clinical functions (Vepari & Kaplan, 2007). Termite hills and their complicated network of tunnels and ventilation systems serve as useful models for constructing buildings in which air quality, temperature and humidity can be regulated efficiently

2.4.3 Insect anatomy and physiology

The word insect was derived from the Latin word insectum, meaning “with a notched or divided body”, literally “cut into sections”, from the fact that insects’ bodies have three parts (Harpe, 2001). Insects are a class of animals within the arthropod group that have a chitinous exoskeleton, a three-part body (head, thorax and abdomen), three pairs of jointed

legs, compound eyes and two antennae. They are among the most diverse groups of animals on the planet: there are more than 1 million described species, which is more than half of all known living organisms. The total number of species is estimated at 6–10 million, and the class potentially represents over 90 percent of the differing animal life forms on Earth. Insects may be found in nearly all environments, although only a small number of species occur in the oceans, a habitat dominated by another arthropod group, the crustaceans (Alexander *et al.,* 2011).

Insects differ in both anatomy and physiology. They have external skeletons (Exoskeleton) which protects the insects and helps to attach their muscles from the inside. Insects take in air through small openings alongside their body parts. Insects have a blood-like fluid haemolymph, which is usually greenish or yellow. The insect haemolymph is pumped around the heart in the abdomen. It flows freely inside the body around the gut, muscles and organs. The insect organs are located in the head, eyes and antennae. Insects have compound eyes, a pair of antennae which allows them to smell and sense the environment. The insect mouth parts vary greatly among species and are related to the type of food consumed by each specie or insect stage (Chapman, 1998). The legs and wings of insects are attached to the thorax. Some insect larvae such as caterpillars have additional legs on the abdomen. The abdomen of an insect serves as its fat reservoirs, the heart and probably, reproductive organs in adults. The life cycle of an insects is divided into several stages; the egg, larvae and adult stages. The number of larvae stages varies among species. The two types of life cycle that exist in insects are complete and incomplete metamorphosis. In complete metamorphosis, the transition phase of the adult is called pupating. The pupa is usually inactive and does not have functional mandibles and becomes active when a mature

adult stage emerges. In incomplete metamorphosis, the larva or nymph hatches from the egg and resembles the adult. Its way of life and diets are similar to those of the adult (Agrodok, 2015).

2.4.4 Classification of insects

There are over 24,000 species of insects and over one million species have been described to date (Delong, 1960). Insects can therefore be classified as follows;

Kingdom -Animalia (all animals)

Phylum -Arthropoda (all arthropods)

Class - Insecta (only the insects)

Order - Diptera (Family - the true flies)

Lepidoptera (family- Butterflies and moths)

Plasmida (Family- Stick insects)

Dictyoptera (Family- Cockroaches, Mantids)

Coleoptera (Family- Beetles)

Orthoptera (Family- grasshoppers)

Hymenoptera (Family- ants, bees and wasps)

Ephemeroptera (Family –mayflies)

2.5 Major Edible Insects and Groups

Beetles *(Coleoptera)* are the most commonly consumed insects in the world with about 31 % consumption rate. The group contains about 40 % of all known insect species. This group is followed by caterpillars (Lepidoptera), especially popular in sub-Saharan Africa with estimated to make up of 18 %. Bees, wasps and ants (Hymenoptera) (these insects are especially common in Latin America) and constitute an estimated 14 % (Alexander *et al.,*

2011). Grasshoppers, locusts and crickets (Orthoptera) constitutes 13 %; cicadas, leafhoppers, planthoppers, scale insects and true bugs (Hemiptera) constitutes 10 %; termites (Isoptera) 3 %; dragonflies (Odonata) 3 %; flies (Diptera) 2 %; and other orders 5 %. Lepidoptera are consumed almost entirely as caterpillars and Hymenoptera are consumed mostly in their larval or pupal stages (Cerritos, 2009).

2.5.1 Coleoptera (beetles)

Coleopteras are one of the largest insect orders. They are commonly called beetles. There are many kinds of edible beetles, including aquatic beetles, wood-boring larvae, and dung beetles (larvae and adults). Ramos *et al*. (2009) listed 78 edible aquatic beetle species, mainly belonging to the Dytiscidae, Gyrinidaeand Hydrophilidae families. Typically, only the larvae of these species are eaten. The most popular edible beetle in the tropics, by far, is the palm weevil, Rynchophorus, a significant palm pest distributed throughout Africa, southern Asia and South America. The palm weevil R. phoenicis is found in tropical and equatorial Africa. In the Netherlands, the larvae of mealworm species from the Tenebrionidae family, such as the yellow mealworm (Tenebriomolitor), the lesser mealworm (Alphitobiusdiaperinus) and the superworm (Zophobasmorio), are reared as feed for reptile, fish and avian pets. They are also considered particularly fit for human consumption and are offered as human food in specialized shops (Van Huis *et al.*, 2013)

2.5.2 Orthoptera (locusts, grasshoppers and crickets)

About 80 grasshopper species are consumed worldwide, and the large majority of grasshopper species are edible. Locusts may occur in swarms, which makes them particularly easy to harvest. In Africa, the desert locust, the migratory locust, the red locust and the brown locust are eaten. However, due to their status as agricultural pests they may

be sprayed with insecticides in governmental control programmes or by farmers. For example, relatively high concentrations of residues of organophosphorus pesticides were detected in locusts collected for food in Kuwait (Saeed *et al.,* 1993). Grasshoppers and locusts are generally collected in the morning when the temperature is cooler (and the insects, being cold-blooded, are relatively immobile).

In Oaxaca, the harvest of chapulines (edible grasshoppers of the genus Sphenarium) only takes place very early in the morning (04:00–05:00 hours) (Cerritos & Cano-Santana, 2008) because chapulinesare too active and difficult to catch during the hotter part of the day (Cohen *et al*., 2009). In the West African nation of Niger, it is not uncommon to find grasshoppers for sale in local markets or sold as snacks on roadsides. Insects are remarkably source of income as researchers found that grasshoppers collected in millet fields fetched a higher price in local markets than the millet itself (van Huis, 2003).

2.5.3 Lepidoptera (butterflies and moths)

Lepidoptera are known as butterflies and moths. They are typically consumed during their larval stages (i.e. as caterpillars), but adult butterflies and moths are also eaten. Indigenous Australians have been reported to eat moths of the cutworm, *Agrotisinfusa* (the Bogong moth) (Flood, 1983) and in the Lao People's Democratic Republic, people have been observed eating hawkmoths (Daphnis and Theretra spp.) after removing the wings and legs (Van Itterbeeck & Van Huis, 2012). Nevertheless, the practice is limited. The mopane caterpillar (*Imbrasia belina*) are the most popular and economically important caterpillar consumed. Endemic to the mopane woodlands in Angola, Botswana, Mozambique, Namibia, South Africa, Zambia and Zimbabwe, the caterpillar's habitat extends over about 384 000 km^2 of forest (Nyoka, 2003). An estimated 9.5 billion mopane caterpillars are

harvested annually in southern Africa, a practice worth US$85 million (Ghazoul, 2006). In Asia, the bamboo caterpillar (*Omphisa fuscidentalis*), is a popular food that is being promoted by the Thai Department of Forestry of the Ministry of Agriculture and Cooperatives as an increasingly viable source of income (Yhoung-Aree & Viwatpanich, 2005).

2.5.4 Hymenoptera (wasps, bees and ants)

Ants are highly sought-after delicacies in many parts of the world. They also render important ecological services, including nutrient cycling, and serve as predators of pests in orchards, although negative effects are also reported (Del Toro *et al*., 2012). The weaver ant (*Oecophylla* spp.) is used as a biological control agent in various crops, such as mangoes (Van Mele, 2008), and the larvae and pupae of the reproductive form (queen brood), also called ant eggs, constitute a popular food in Asia. In Thailand they are sold in cans. Shen & Ren, (2006) reported that the black weaver ant (*Polymachis dives*) is widely distributed in subtropical southeast China, Bangladesh, India, Malaysia and Sri Lanka. It is used as a nutritional ingredient and processed into various tonics or health foods available on the Chinese market. In Japan, the larvae of yellow jacket wasps, are commonly consumed. During the annual Hebo Festival, food products made from the larvae of the wasps are popular delicacies (Nonaka *et al.,* 2008).

2.5.5 Heteroptera (true bugs), suborder of Hemiptera

Pentatomid bugs are eaten widely throughout sub-Saharan Africa, particularly in southern Africa in the Republic of Sudan, the pentatomid, *Agonoscelisversicolor*, a pest of rainfed sorghum that causes considerable damage, is eaten roasted. Oil is also derived from these insects and is used in preparing foods and for treating scab disease in camels (Nyoka, 2003).

Most pentatomids consumed as food, however, live in water. The famous *Mexicancaviar,* ahuahutle, is composed of the eggs of at least seven species of aquatic Hemiptera (the Corixidae and Notonectidae families); these insects have formed the backbone of aquatic farming, or aquaculture, in Mexico for centuries. The semi-cultivationof these species is simple and inexpensive because it can be undertaken using traditional local practices (Parsons, 2010).

2.5.6 Isoptera (termites)

The most commonly eaten termite species are the large Macrotermes species. The winged termites emerge after the first rains fall at the end of the dry season, from holes near termite nests. Termites are used as food in various cultures. These insects are rich in protein and fat (Igw*et al.,* 2011). Termites are eaten raw, fried, roasted, or prepared in various ways which may be suiTable for consumption (Agrodok, 2015).

2.6 Insect optimization

Climate changes have significant impact on natural environment, regardless of their primordial cause. They also influence forest ecosystems including tree species and living components. Insects groups are among the greatest animals of importance, including species that detrimental to forest wellbeing as regard to forest management.Insects are ectothermic group of animals thus; they are highly dependent on thermal conditions of the surrounding environment. Climate conditions are basic factors that affect insect forms and distribution range (Solomon, 2007). Any climate changes that is not neutral for insects' assemblages. The impact of climate change on forest insects may be reflected, for example, by affecting their range, phenology, activity, number of generations and winter survival. Among the present theories of climate changes, average temperature increase theory

dominates (Solomon, 2007). Most of the several dozens of predictive models indicate that average temperature can increase by 1.7–5.3 °C, as a result of doubling CO_2 combustion within the next 60–100 years. A change of 2.3 °C ismost often mentioned which means an increase by 0.3 °C a decade. As opposed to temperature, humidity is characterized by higher variability. It is difficult to indicate a distinct trend within humidity as it is with regard to temperature

Climate change is not favourable for most insects because insects as ectoderms and are bound to respond to the temperature change, and different species respond differently depending on their specific physiological and ecological traits, seasonal cycle, and trophic relations, among others. Insect responses to climate warming can be divided into six categories: changes in ranges, abundance, phenology, morphology, physiology, and behavior. These changes can be easily observed and recorded. However, temperature, humidity, photoperiods and nutrient availability plays a significant role in influencing insect life histories and population dynamics (Woods *et al*., 2003) all these bring about substansive changes to insect's survival either by causing local increases or declines in growth, development and reproduction.

2.6.1 Effects of temperature in insect's development

Insects are capable of sensing and responding to temperature variation (Blum, 1985; Chown & Nicolson, 2004). The body temperature of most insects is linked to changes in ambient temperature. Most insects are poikilothermic, that is their body temperature more or less directly varying with environmental temperature, and thus heat is the force driving the rate of growth and development when food is unlimited. A rise in temperature, within a favorable range, will speed up the metabolism of an insect and consequently increase its

rate of development. Each species and each stage in the life history may develop at its own rate in relation to temperature. Thus, physiologically, a measure of the amount of heat is required over time for an insect to complete development or a stage of development, is more meaningful as a measure of development time than age in calendar time. Knowledge of relationships between temperature development and the use of physiology allow comparison of the life cycles and/or fecundity of pest species in the same system and prediction of the larval feeding periods, generation length, and time of adult emergence under variable temperature conditions that exist in the field. Such predictions are especially important for pest insects, as control measures must be timed carefully to be effective (Regniere *et al.,* 2012).

Physiological time is the cumulative product of total development time (in hours or days) multiplied by the temperature (in degrees) above the developmental (or growth) threshold, or the temperature below which no development occurs. Thus, physiological time is commonly expressed as day-degrees (D°) or hour- degrees (h°). Normally, physiological time is estimated for a species by rearing a number of individuals of the life-history stage(s) of interest under different constant temperatures in several identical growth cabinets (Regniere *et al.,* 2012).

In practice, the application of laboratory estimated physiological time to natural populations may be complicated by several factors. Under fluctuating temperatures, especially if the insects experience extremes, growth may be retarded or accelerated compared with the same number of day-degrees under constant temperatures. Furthermore, the temperatures actually experienced by the insects, in their often sheltered microhabitats on plants or in soil or litter, may be several degrees different from the temperatures recorded at a

meteorological station even just a few meters away. Insects may select microhabitats that ameliorate cold night conditions or reduce or increase daytime heat. Thus, predictions of insect life-cycle events based on extrapolation from laboratory to field temperature records may be inaccurate (Regniere *et al.,* 2012). For these reasons, the laboratory estimates of physiological time should be corroborated by calculating the hour-degrees or day degrees required for development under more natural conditions, but using the laboratory estimated developmental threshold, as follows:

i. Place newly laid eggs or newly hatched larvae in their appropriate field habitat and record temperature each hour.

ii. Estimate the time for completion of each instar by discarding all temperature readings below the developmental threshold of the instar and subtracting the developmental threshold from all other readings to determine the effective temperature for each hour (or simply subtract the development threshold temperature from the daily average temperature). Sum the degrees of effective temperature for each hour from the beginning to the end of the stadium. This procedure is called thermal summation.

iii. Compare the field-estimated number of hour-degrees (or day-degrees) for each instar with that predicted from the laboratory data. If there are discrepancies, then microhabitat and/or fluctuating temperatures may be influencing insect development or the developmental zero read from the graph may be a poor estimate of the developmental threshold (Regniere *et al.,* 2012).

Another problem with laboratory estimation of physiological time is that insect populations maintained for lengthy periods under laboratory conditions frequently undergo acclimation

to constant conditions or even genetic change in response to the altered environment or as a result of population reductions that produce genetic "bottle-necks". Therefore, insects maintained in rearing cages may exhibit different temperature development relationships from individuals of the same species in wild populations. For all of the above reasons any formula or model that purports to predict insect response to environmental conditions must be tested carefully for its fit with natural population responses (Woods *et al*., 2003)

2.6.2 Effects of photoperiods in insect development

Many insects, perhaps most, do not develop continuously all year round, but avoid some seasonally adverse conditions by a resting period or migration (Woods *et al*., 2003). Summer dormancy (aestivation) and winter dormancy (hibernation) provide two examples of avoidance of seasonal extremes. The most predictable environmental indicator of changing seasons is photoperiod (the length of the daily light phase or, more simply, day length). Near the equator, although sunrise to sunset of the longest day may be only a few minutes longer than on the shortest day, if the period of twilight is included then total day length shows more marked seasonal change (Brian & Brad, 2019). The photoperiod response is to duration rather than intensity and there is a critical threshold intensity of light below which the insect does not respond; this threshold is often as dim as twilight, but rarely as low as bright moonlight.

Many insects appear to measure the duration of the light phase in the 24 hours period, and some have been shown experimentally to measure the duration of dark. Others recognize long days by light falling within the "dark" half of the day. Most insects can be described as "long-day" species, with growth and reproduction in summer and with dormancy commencing with decreasing day length. Others show the reverse pattern, with "short-day"

(often fall and spring) activity and summer aestivation. In some species the life-history stage in which photoperiod is assessed is in advance of the stage that reacts, as is the case when the photoperiodic response of the maternal generation of silkworms affects the eggs of the next generation (Woods *et al*., 2003).

The ability of insects to recognize seasonal photoperiod and other environmental cues requires some means of measuring time between the cue and the subsequent onset or cessation of diapause. This is achieved through a "biological clock" which may be driven by internal (endogenous) or external (exogenous) daily cycles, called circadian rhythms. Interactions between the short time periodicity of circadian rhythms and longer-term seasonal rhythms, such as photoperiod recognition, are complex and diverse, and have probably evolved many times within the insects (Woods *et al*., 2003).

2.6.3 Effects of humidity in insect development

The high surface area and volume ratio of insects means that loss of body water is a serious hazard in a terrestrial environment, especially a dry one. Low moisture content of the air can affect the physiology and thus the development, longevity, and oviposition of many insects. Air holds more water vapor at high than at low temperatures (Woods *et al*., 2003).

The relative humidity (RH) at a particular temperature is the ratio of actual water vapor present to that necessary for saturation of the air at that temperature. At low relative humidities, development may be retarded, for example in many pests of stored products; but at high relative humidities or in saturated air (100 % RH), insects or their eggs may drown or be infected more readily by pathogens. The fact that stadia may be greatly lengthened by unfavorable humidity has serious implications for estimates of development times, whether calendar or physiological time is used. The complicating effects of low, and

sometimes even high, air moisture levels should be taken into account when gathering such data (Woods *et al*., 2003)

2.6.4 Effects of mutagens and toxins

Stressful conditions induced by toxic or mutagenic chemicals may affect insect growth and form to varying degrees, ranging from slight phenotypic modifications at one end to death at the other extreme of the spectrum. Some life-history stages may be more sensitive to mutagens or toxins than others, and sometimes the phenotypic effects may not be easily measured by crude estimates of stress, such as percentage survival (Woods *et al*., 2003). One sensitive and efficient measure of the amount of genetic or environmental stress experienced by insects during development is the incidence of fluctuating asymmetry, or the quantitative differences between the left and right sides of each individual in a sample of the population. Insects are usually bilaterally symmetrical if grown under ideal conditions, so the left and right halves of their bodies are mirror images (except for obvious differences in structures such as the genitalia of some male insects). If grown under stressful conditions, however, the degree of asymmetry tends to increase (Liebsch *et al*., 2011).

The measurement of fluctuating asymmetry has many potential uses in theoretical and economic entomology and in assessment of environmental quality. For example, it can be used as an indicator of developmental stability to determine the effect on non-target organisms of exposure to insecticides or vermicides, such as avermectins. Bush flies (*Muscavetustissima*) breeding in the dung of cattle treated for nematode control with Avermectin B1 are significantly more asymmetric for two morphometric wing characters than flies breeding in the dung of untreated cattle (Sukhodolskaya *et al.,* 2019). For

example, water quality has been assessed by comparing the asymmetry in aquatic insects reared in polluted and clean water. In industrially polluted waters, particular bloodworms (larvae of chironomid midges) may survive but often exhibit gross developmental abnormalities.

However, at lower levels of pollutants, more subtle effects may be detected as deviations from symmetry compared with clean-water controls. In addition, measures of developmental effects on non-target insects have been used to assess the specificity of biocides prior to marketing. The technique is not completely reliable, with doubts having been raised about interpretation (variation in response between different organ systems measured) and concerning the underlying mechanism causing any responses measured (Kathren, 2002).

2.6.5 Biotic effects in insect's development

In most insect orders, adult size has a strong genetic component and growth is strongly determinate. In many Lepidoptera, for example, final adult size is relatively constant within a species; reduction in food quality or availability delays caterpillar growth rather than causing reduced final adult size, although there are exceptions. In contrast, in flies that have limited or ephemeral larval resources, such as a dung part or temporary pool, cessation of larval growth would result in death as the habitat shrinks. Thus larval crowding and/or limitation of food supply tend to shorten development time and reduce final adult size. In some mosquitoes and midges, success in short-lived pool habitats is attained by a small proportion of the larval population developing with extreme rapidity relative to their slower siblings (Hu *et al*., 2012). In pedogenetic gall midges crowding with reduced food supply terminates larva-only reproductive cycles and induces the production of adults, allowing

dispersal to more favorable habitats. Food quality appears important in all these cases, but there may be related effects, for example as a result of crowding. Clearly, it can be difficult to segregate out food effects from other potentially limiting factors. In the California red scale, Aonidiellaaurantii (Hemiptera: Diaspididae), development and reproduction on orange trees is fastest on fruit, intermediate on twigs, and slowest on leaves. Although these differences may reflect differing nutritional status, a microclimatic explanation cannot be excluded, as fruit may retain heat longer than the relatively smaller-volumed stems and leaves, and such slight temperature differences might affect the development of the insects (Tang *et al*., 2014).

The effects of crowding on development are well understood in some insects, as in locusts in which two extreme phases, termed solitary and gregarious differ in morphometrics, color, and behavior. At low densities locusts develop into the solitary phase, with a characteristic uniform-colored “hopper” (nymph), and large-sized adult with large hind femora. As densities increase, induced in nature by high survivorship of eggs and young nymphs under favor- able climatic conditions, graded changes occur and a darker-striped nymph develops to a smaller locust with shorter hind femora (Barrette *et al*., 2009). The most conspicuous difference is behavioral, with more solitary individuals shunning each other’s company but making concerted nocturnal migratory movements that result eventually in aggregations in one or a few places of gregarious individuals, which tend to form enormous and mobile swarms. The behavioral shift is induced by crowding, as can be shown by splitting a single locust egg pod into two: rearing the offspring at low densities induces solitary locusts, whereas their siblings reared under crowded conditions develop into gregarious locusts. The response to high population density results from the integration of several cues,

including the sight, touch, and sometimes the odor (pheromone) of conspecifics, which lead to endocrine and neuroendocrine (ecdysteroid) changes associated with developmental transformation (Wang *et al*., 2011).

Under certain circumstances biotic effects can override growth factors. Across much of the eastern USA, 13- and 17-year periodic cicadas (*Magicicada* spp.) emerge highly synchronously. At any given time, nymphal cicadas are of various sizes and in different instars according to the nutrition they have obtained from feeding on the phloem from roots of a variety of trees. Whatever their growth condition, after the elapse of 13 or 17 years since the previous emergence and egg-laying, the final molt of all nymphs prepares them for synchronous emergence as adults (Yang *et al*., 2014).

2.7 Moth caterpillar (*Cirina forda)*

Cirina forda, is a moth of the family *Saturniidae*. The species was first described by John O. Westwood in 1849. It is found in most African countries including Ghana, Nigeria, Zimbabwe, the Democratic Republic of the Congo and South Africa. Adults are pale creamy brown with a small darker spot on each hindwing but lacking true eyespots. It undergoes a complete metamorphosis. The larvae feed on *Vitellaria paradoxa*. The larvae are consumed (entomophagy) in Nigeria and the Democratic Republic of the Congo. Pupation takes place in soft soil or sand at the base of the host plant.

Cirina forda is commonly known as the Pallid emperor moth or shear defoliator. It is a pest of the sheabutter tree *Vittelaria paradoxa* (Sapotaceae) indigenous in Nigeria, and part of southern, east, west and central Africa. They are usually available during the rainy season. It serves as a source of food to human and as pest to economic trees. It is one of the most widely marketed edible insect species in Nigeria (De foliart, 2002) and widely consumed

during the most food deficient period of the year (May to June). The population dynamics of the insect has been well studied to obtain a more predicTable harvest with consequent economic stability.

The edible larvae of *Cirina forda* insect has a wide acceptability as a food source and also serves as an important item of commerce in such Nigerian states like Oyo, Kwara, Kogi, Niger and Kaduna where it has become the most important and markeTable insect (Ande,1991; Fasoranti and Ajiboye,1993). The larvae of this insect resemble silk worm caterpillars but they do not spin cocoons. Instead, they dig into the soil at the base of the host tree to pupate. It is a popular food item in Nigeria, its name in various dialects includes Manimani (Nupe), Obubu/Egwu (Igbo), Suzza/Manimani (Hausa) and Kanni Wole (Yoruba) 'konni means *Cirina forda* while 'wole' means to enter soil. Fasoranti and Ajiboye (1993), in their paper titled 'Edible insects in Kwara State', recognized the potential and the level of acceptance of *Cirina forda* as a popular food source by observing that, it is the most popularly accepted food insect in the Western region of Nigeria. They further described it as the most important and marketable insects in Kwara State (Ande, 1991). The larvae are excellent source of protein, vitamins and minerals for human and livestock consumption (Fasoranti & Ajiboye, 1993) and also a source of income. Apart from being a widely acceptable food source, a number of factors are known to enhance the availability of the larvae of the insect. Such factors include its capability for artificial rearing the short-lived larval stage, and the high conversion rate (Ande, 1991).

Figure 2.1: *Cirina forda*

Source: Omotosho, 2006.

2.7.1 Classification of *Cirina forda*

Cirina forda can be classified as follows;

Kingdom - Animalia

Phylum - Arthropoda

Class - Insecta

Order - Lepidoptera

Family - Saturniidae

Genus - Cirina

Species – *Cirina forda* (Moth caterpillar)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

All chemicals and reagents used were of analytical grade manufactured by British Drug House (BDH) limited, England and Sigma Aldrich Chemical Company Incorporation Milwaukee, wisconson USA.

Assay kits for total protein, creatinine, bilirubin, chloride, albumin and urea were products of spectrum diagnostics,Hannover, Germany while assay kits for potassium & sodium,were products of Teco diagnostics , Lakeview Avenue Anabeim USA while Alkaline phosphate, Aspartate transaminase were products of Agappe diagnostics Switzerland.

3.1.2 Equipment

Amino acids analyzer (applied biosystem PTH amino acid analyzer model 120A PTH, USA), mindray Auto Hematology analyser BC- 5300 model, Atomic absorption spectrometer -6800 shimadzu, shimdazu UV-spectrometer UV-1800, Moisture extraction oven (Gallenkamp ,size 2, brainweigh B, UK) Hot plate (Biotech India), Genway model 6000 electronic spectrometer), Water bath (Surrey GU 185TA,UK), Rotary evaporator (RE500 Yamato scientific America Incorporations USA, USA), Electronic compact Scale(Labtech BL10001), Water bath (light water, surrey GU185TA, UK),Medium scale grinding engine (Henry west G160, 5.5HP), Crown star thermostat-heating element (model: MC- HP1000) with power of 230 50/60Hz 1000w, Rechargeable LED light (LED-9087B).

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3.2 Methods

3.2.1 Collection of sample materials

3.2.1.1 Emperor moth (*Cirina forda*)

Freshly laid egg clutches of *Cirina forda* as well as matured *Cirina forda* larvae were collected from Lavun village in Bida Local Government Area of Niger state.

3.2.1.2 Cassava flakes (*Manihot esculenta*)

Already processed cassava flakes (*Manihot esculenta*) sample was obtained from Bosso market, Bosso Local Government, Minna, Niger State.

3.2.1.3 Soybean (*Glycine max*)

The Soybean (*Glycine max*) sample was obtained from bosso market, Bosso Local Government, Minna, Niger State.

3.2.2 Specimen identification

The *Cirina forda* eggs and matured larvae samples were identified by a zoologist in the department of animal biology, Federal University of Technology, Minna.

3.2.3 Optimization of *Cirina forda*

Optimization was carried out as summerised below.

3.2.3.1 Foliage collection for *Cirina forda*

Fresh leaves of *Vittelaria paradoxa* were collected from Mariam Babangida girls' college, Minna, Niger State, between 8:00 a.m and 12:00 p.m daily. Larvae from old and dried leaves were detached and carefully placed on fresh twigs when they were dormant, but when the larva became more active, old twigs were rather carefully placed on fresh twigs in such a way that the larvae could easily migrate from dried leaf to fresh ones.

3.2.3.2 Exposure of *Cirina forda* to environmental factors

The larvae were exposed to combined optimum conditions of temperature at 28 ^{o}C for 15 hours everyday, relative humidity of 96 % and photoperiod of 18 hours of Light: 6 hours of Darkness (18HL:6HD) daily for a period of two months.

The larvae were placed in an iron frame box having one side covered with perforated carton and three sides covered with towels. Water was sprayed occasionally on the towels to generate humidity. Thermostat-heating element (crown star), model: MC- HP1000 with power of 230 50/60Hz 1000w was used to generate temperature of 28^{o}C for 15 hours daily. A rechargeable LED light (LED-9087B) was used to provide light during dark hours.

3.2.4 Sample preparation

Samples were prepared following the procedures described by Onwuka (2005).

3.2.4.1 Insect preparation

After the *Cirina forda* eggs were raised under various optimal conditions in the Department of animal biology, FUT, Minna, the matured optimised *Cirina forda* and non-optimised *Cirina forda* larvae were dried under normal room temperature and stored in plastic containers for analysis.

3.2.4.2 Soy flour preparation

The dry soybean samples were washed, air dried, roasted and grinded to powder using a mechanical grinding machine.

3.3 Proximate Analysis

The moisture, total ash, crudefibre, crude protein, crude lipid and total carbohydrate contents were determined using the methods described by Onwuka (2005).

3.3.1 Determination of moisture content

The principle of moisture content determination is through a thermo-gravimetric method, that is, by loss on drying, in which the sample is heated and the weight loss due to evaporation of moisture is recorded as described by Onwuka (2005).

Procedure

Two grams (2.0 g) of the sample was accurately weighed in clean, dried crucible. The crucible with its content was transferred into an oven and dried at a temperature of 80 ^{o}C for 2 hours and 100 ^{o}C for another 4 hours until a constant weight was obtained. The sample was allowed to stand in a desiccator for 30minutes to cool. After cooling, the crucible was re-weighed. The percentage moisture was calculated using equation 1:

$$Moisture\ Content\ (\%) = \frac{W2 - W3}{W2 - W1} \times 100 \qquad \textbf{Equation 1}$$

Where; W_1 = Initial weight of empty crucible

W_2 = Initial weight of empty crucible + Sample before drying

W_3 = Final weight of crucible + Sampleafter drying

3.3.2 Determination of ash content

The principle of ashing involves burning off organic matter to determine the inorganic matter remaining. Heating is carried out in two stages; firstly to remove the water present and to char the sample thoroughly; and finally ashing at 550 ^{0}C in a muffle furnace, the weight of ash thus obtained is expressed in terms of percentage.

Procedure: Clean empty crucible was placed in a muffle furnace at 550 ^{o}C for an hour, cooled in desiccator and then the weight of empty crucible was noted (W_1). Two grams of the sample was placed in the crucible and weighed as (W_2). The crucible with its content

was placed over a burner, until its content was charred. Then, the crucible with the charred content was moved into a muffle furnace for ashing at 550 ^{o}C for 2 hours. The appearance of gray white ash indicates complete oxidation of all organic matter in the sample. After ashing the crucible was cooled and weighed (W_3). Percentage ash was calculated using the formula in equation 2.

$$Ash\ Content\ (\%) = \frac{Weight\ of\ Ash}{Weight\ of\ Sample} \times 100 = \frac{W3 - W1}{W2 - W1} \times 100 \qquad \textbf{Equation 2}$$

Where:

W_1=weight of empty crucible,

W_2= weight of crucible + Sample before ashing and

W_3=weight of crucible + sample after ashing

3.3.3 Determination of crude protein content

The principle of crude protein content determination can be determined following the titration techniques of kjeldal. The principle is based on digestion of organic matter with sulfuric acid in the presence of a catalyst, rendering the reaction product alkaline the distillation and titration of the liberated ammonia, calculation of the nitrogen content, multiplication of the result by the conventional factor 6.25 to obtain the crude protein content.

Proceedure

Protein content in sample was determined by kijeldahl method. 0.25 g of dried sample was taken in digestion flask, and 6 ml of concentrated H_2SO_4 and a speck of kjeldahl catalyst (mixture of 10 g Na_2SO_4+5 g CuSO4+ 0.05 g selenium) were added. The flask was swirled in order to mix the contents thoroughly then digested on a digestion block till the mixtures became clear (colourless or greenish in color). The digest was cooled and transferred to a

100 ml volumetric flask and the volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham distillation apparatus. Ten milliliters of digest was introduced in the distillation tube then 10 ml of 40 % NaOH was gradually added through the same way. It was left to distill for 10 min and the NH_3 produced was collected as NH_4OH in a conical flask containing 5ml of 4 % boric acid solution with few drops of methyl red indicator. During distillation yellowish color appeared due to NH_4OH. The distillate was then titrated against standard 0.1 N HCI solution till the appearance of pink color. Percentage crude protein content of the insects and grain sample was calculated using the following formula in equation 3:

$$Nitrogen\ Content\ (\%) = \frac{(S-B) \times N \times 0.014 \times D}{Weight\ of\ Sample \times V} \times 100 \qquad \textbf{Equation 3}$$

Where;

S = Sample titration reading B = Blank titration reading

N = Normality of HCI D = Dilution of sample after digestion

V = Volume taken for distillation 0.014 – Milli-equivalent weight of Nitrogen

% Crude Protein = 6.25 x %Nitrogen (Conversion factor)

3.3.4 Determination of crude fat

The principle of crude fat content determination is determined mainly by solvent extraction method. Fat is extracted with a mixture of diethyl ether and petroleum ether. The extract containing the fat is dried and expressed as percent fat by weight.

Proceedure

Crude fat content of insect sample was determined using the method described by AOAC, (1990). Five gram (5.0 g) of sample was taken into a thimble of known weight (W_1). The

weight of the thimble and sample was taken (W_2). The thimble with the sample was placed in a Soxhlet extractor. 300ml of petroleum ether was poured into a 500ml round bottom ground joint flask, which was placed on a heating mantle. The heating and extraction was carried out for 24 hours, after which the thimble with content was removed dried in an oven at 50 ^oC for 24 hours, cooled in a desicator and weighed (W_3). The percentage crude fat content of each sample was calculated using the formula equation 4:

$$Crude\ Fat\ Content\ (\%) = \frac{W2 - W3}{W2 - W1} \times 100 \qquad \textbf{Equation 4}$$

Where: W_1=weight of empty crucible, W_2= weight of crucible + Sample before ashing and W_3=weight of crucible + sample after ashing

3.3.5 Determination of crude fiber content

The principle of crude fiber determination is determined gravimetrically after chemical digestion and solubilization of test sample. The principle is based on acid/alkali treatment, oxidative hydrolytic degradation of cellulose and lignin and filtration. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content.

Proceedure

Two gram (2.0 g) of sample was defatted with petroleum ether; boiled under reflux for 30minutes with 200 ml of a solution containing 1.25 % of H_2SO_4 solution. The solution was filtered through several layers of cheese cloth on fluted funnel, washed with boiling water until the washings were no longer acidic then the residue was transferred into a beaker and boiled for 30 minutes with 200 ml of solution containing 1.25 % NaOH solution. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, then dried in an electric oven and weighed after which it was incinerated at 550 ^oC

for 30 mins, cooled and reweighed. The loss in weight was expressed as a percentage of initial weight of the sample according to equation 5:

$$Crude\ Fibre\ Content\ (\%) = \frac{W2 - W3}{W2 - W1} \times 100 \qquad \textbf{Equation 5}$$

Where: W_1=weight of empty crucible, W_2= weight of crucible + Sample before ashing and W_3=weight of crucible + sample after ashing.

3.3.6 Determination of carbohydrate

The principle of carbohydrate content determination can be measured by hydrolyzing polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide. Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm.

Proceedure

The total amount of crude protein, crude fat, moisture and ash of each of the samples was added and subtracted from 100. The value obtained was the percentage carbohydrate content of the samples as described by Nielsen, (2002) % Carbohydrate = 100 – (% moisture + % ash + % Protein + % Fat)

3.4 Determination of minerals

The principle of mineral contents determination can be determined using atomic absorption spectroscopy. The principle is based on electronic transitions of outer shell electrons. The energy change associated with a transition between two energy levels is related to the wavelength of the absorbed radiation. Thus for a given transition between two energy states radiation of a discrete wavelength is either absorbed or emitted. Each element has a unique

electronic structure and therefore it has a unique set of energy levels. Consequently, it absorbs or emits radiation at specific wavelengths. Each spectrum is therefore like a "fingerprint" that can be used to identify a particular element.

Proceedure

The mineral contents in each sample were determined according to the methods described by Nielsen, (2002). Two gram (2.0 g) of the samplewas weighed into a crucible, and samples were allowed to ash at 550 ^{o}C for about 2 hours. The ash was transferred into a 250 ml beaker and 15ml of concentrated hydrochloric acid and 5ml of concentrated nitric acid were added. The beaker was placed on a hotplate to heat to about 100 ^{o}C until the acid was evaporated to dryness. Then, 10 ml of distilled water was added to the beaker and sample was filtered into a 100 ml volumetric flask and made up to mark. The content of the digested sample was analyzed on a Shimadzuatomic absorption spectrophotometer – 6800.

3.5 Anti-nutrients

The anti-nutrients determined in each sample were tannins, phytates, alkaloids, saponins, cyanogenic glycosides and oxlates.

3.5.1 Determination of tannin content

Tannins contents were determined according to the methods described by Onwuka, (2005). 0.2 g of sample was measured into a 50 ml beaker. 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at 80 ^{O}C for 1 hour. It was shaken thoroughly to ensure a uniform mixture. The extract was filtered using a double layered Whitman No.41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml Folin-Denis reagent and 10 ml of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min for the

development of a bluish-green colour. The absorbance of the tannic acid standard solutions as well as samples was read after colour development using Jenway model 6000 Electronic spectrophotometer at a wavelength of 760 nm. The tannin content was calculated as follows: %Tannin = An/As XCx100/W x Vf

Where An = absorbance of test sample, As = absorbance of standard solution, C = concentration of standard solution, W = Weight of sample, Vf = Total Volume of extract.

3.5.2 Determination of phytates

The phytate content was determined using the indirect colorimetric method of Lolas & Markakis, (1975) as described by Essien & Akpan, (2014). Two gram (2.0 g) of sample was weighed into a 250 ml conical flask, and soaked in a conical flask of 100 ml of 2 % concentrated HCl for 3 hours. The mixture was filtered and 50 ml of filtrate was placed in a 250 ml beaker and 107 ml of distilled water was added. 0.3 % ammonium thiocyanate (10 ml) was added to the sample as indicator and titrated with iron III chloride solution which contained 1.95 mg iron per ml. Titration continued until a brownish yellow colour that persisted for 5 minutes was observed.

3.5.3 Determination of total alkaloid

The method described by Harrborne (1976) was used in determining alkaloids contents. Exactly 0.5 g of the sample was dissolved in a mixture of 96 % ethanol and 20 % H_2SO_4 (1:1). Then 1 ml of the solution was filtered using a Whitman filter paper followed by addition of 5 ml of 60 % tetraoxosulphate (VI) H_2SO_4, and allowed to stand for 5min. 5ml of formaldehyde 0.5 % was added and allowed to stand for 3hours. The absorbance was read at 565 nm. Vincristine was used as standard. The extinction coefficient E_{296}, ethanol {ETOH} = 15136 $M^{-1}cm^{-1}$).

3.5.4 Determination of oxalates

Oxalate in the sample was determined by permanganate titrimetric method as described by Oke, (1966). Approximately 2.0 g of each sample was suspended in 190 ml of distilled water in 250 ml volumetric flask, 10 ml of 6 M HCI was added and the suspension digested at 100 ^{o}C for 1 hr, cooled, then made to the mark before filtration. Approximately 125 ml duplicate portion of the filtrate was measured into beakers and 4 drops of methyl red indicator added. This was followed by the addition of concentrated NH_4OH solution drop wise until the solution changed from salmon pink to a faint yellow colour (pH 4-4.5). Each portion of the filtrate was then heated to 90^{o}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90 ^{o}C and 10ml of 5 % $CaC1_2$ solution was added gradually while being stirred constantly. After heating, it was cooled and left overnight at 5 ^{o}C. The solution was then centrifuged at 2500 rpm for 5mins, the supernatant was decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for 30secs. The calcium oxalate content is calculated using the formula in equation 6 below:

$$Oxalate\ Content\ (mg/100g) = \frac{T \times Vme\ (Df) \times 10^5}{ME \times Mf} \times 100 \qquad \textbf{Equation 6}$$

Where;

T = titre value of $KMnO_4$ (ml),

Vme = volume-mass equivalent (1 cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid)

Df = dilution factor V_T/A (2.5 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml),

ME = Molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and

Mf = Weight of sample used.

3.5.5 Determination of saponins

Saponin content was determined according to the method described by Oloyede, (2005). 0.5 g of each sample was added to 20 ml of 1 N HCl and was boiled for 4hour. After cooling, it was filtered and 50ml of petroleum ether was added to the filtrate of the ether layer and evaporated to dryness on hot plate. Afterwards, 5ml of acetone ethanol was added to the residue, 0.4 ml of each was then transferred into 3 different test tubes. 6.0 ml of ferrous sulphate reagent was added into each of the 3 test tubes followed by 2 ml of concentrated H_2SO_4. These were thoroughly mixed and left to stand for 10 min and the absorbance taken at 490nm. Standard saponin was used to establish the calibration curve.

3.5.6 Determination of cyanogenic glycosides

Cyanide content was determined by alkaline picrate method as described by Onwuka, (2005). Exactly 5 g of each sample was dissolved in 50 ml of distilled water in a conical flask and allowed to stand overnight to extract cyanide. The extract was filtered and the filtrate was used for cyanide determination.To 1 ml of sample filtrate, 4 ml of alkaline picrate was added in a corked test tube and incubated in a water bath for 5 minutes. After colour development (reddish brown colour) the absorbance was read at 490 nm. The cyanide content was extrapolated from a cyanide standard curve.

3.6 Amino acid analysis

The Amino Acid profile in the sample was determined using method described by Benitez, (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer (Model120A PTH amino acid Analyzer).

3.6.1 Defatting Sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. 500 mg of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus (AOAC, 2006).

3.6.2 Nitrogen Determination

Approximately 115 mg of insect sample was weighed, wrapped in Whitman filter paper (No.1) and put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10ml of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2 % boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was titrated against a 0.01 N hydrochloric acid until a grey coloured end point was reached. The percentage nitrogen content was calculated using equation 7:

$$Nitrogen\ Content\ (\%) = \frac{(a-b) \times 0.01 \times 14 \times V}{W \times C} \times 100 \qquad \textbf{Equation 7}$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100 ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10 ml)

14. = Nitrogen constant in mg.

0.01 = normality of Hydrochloric acid

3.6.3 Hydrolysis of the sample

A gram of the defatted sample was weighed into glass ampoule. Exactly 7 ml of 6 N HCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis example methionine and cystine. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ^{o}C± 5 ^{o}C for 22 hours. The ampoule was allowed to cool, broken open at the tip and the content was filtered to remove the humins.The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottle, which was kept in the freezer at 4 ^{o}C for further analysis.

3.6.4 Loading of the hydrolysate into analyzer

Five microlitre of sample was dispensed into the cartridge of the analyser. The TSM analyzer is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 76 minutes.

3.6.5 Calculation of amino acid values from chromatogram peaks

The net height of each peak produced by chart recorder of TSM (each representing an Amino acid) was measured. Area of each peak was then obtained by multiplying the height with the width at half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using formula:

$$Norleucine\ Equivalent\ (NE) = \frac{Area\ of\ Norleucine\ Peak}{Area\ of\ each\ Amino\ Acid} \qquad \textbf{Equation 8}$$

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

Where S_{std} =NE_{std} x Molecular weight x μMAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = NH x W at NH/2 x Sstd x C

Where $C = \frac{Dilution \times \frac{16}{NH} \times W\ (nleu)}{Sample\ Wt\ (g) \times N(\%) \times Loaded\ Vol. \times 10}$ **Equation 9**

Where: NH = Net Height

W = Width @ half height

Nleu = Norleucine

3.6.6 Determination of amino acid score or values

The amino acid score (AAS) for each essential amino acid was calculated based on FAO/WHO (1985) suggested patterns of amino acid requirements for preschool children (2-5 years). The amino acid score for each amino acid was calculated using the formula.

$$AAS = \frac{mg\ of\ Amino\ Acid\ in\ 1g\ of\ test\ Protein}{Amino\ Acid\ Ref.\ for\ Preschool\ Children\ (mg/g)} \qquad \textbf{Equation 10}$$

The limiting amino acid for each test protein was obtained as the amino acid with the lowest amino acid score in each protein (Nielsen, 2002)

3.7 Feed Formulation

Six experimental diets were formulated using soybean, optimised *Cirina forda* and non-optimised *Cirina forda* as sources of protein. Each diet contained 10 % and 20 % protein with exception of the optimised *Cirina forda* which contained only 10 % protein. The diets were prepared following the proportions as shown in Table 3.1. The components of the diets were mixed manually, pelletized using a mechanical pelletizing machine into small pellets and stored in a well labeled polyethylene bag

Table 3.1: Composition of *Soybean*, Optimised and Non-optimised *Cirina forda* diets (per kg diet)

Sample	A - *Control diet*	B- *Soybean* (10 % protein)	C- *Soybean* (20 % Protein)	D- Optimised *Cirina forda* (10 % Protein)	E- Non-optimised *Cirina forda* (10 % protein)	F- Non-optimised *Cirina forda* (20 % protein)
Weight of sample	650	248.63	497.27	180.67	194.78	389.56
Weight of basal diet		401.37	152.73	469.33	455.22	260.44
Vegetable oil	100	100	100	100	100	100
Sucrose	100	100	100	100	100	100
Glucose	45	45	45	45	45	45
Cellulose	50	50	50	50	50	50
Mineral & Vitamin Mix	50	50	50	50	50	50
Methionine	3	3	3	3	3	3
Lysine	2	2	2	2	2	2

3.7.1 Experimental Design

The study was designed to utilize thirty (30) rats were divided into 6 groups of 5 animals each. The rats were randomly distributed into six groups as shown below:

Group A (positive Control): Rats fed with standard *control diet*

Group B (Standard Control): Rats fed with diet containing 10 % *Soybean* protein

Group C (Standard Control 2): Rats fed with diet containing 20 % *Soybean* protein

Group D: Rats fed with diet containing 10 % Optimised *Cirina forda* protein

Group E: Rats fed with diet containing 10 % non-optimised *Cirina forda* protein

Group F: Rats fed with diet containing 20 % non-optimised *Cirina forda* protein

3.7.2 Determination of daily feed intake

The feeding experiments were carried out for 21 days. Initial weights of animals were taken prior to commencement of feed administration experiment and weights of animals were taken on a weekly basis. Feed intake was equally measured on a daily basis.The daily feed intake was determined by weighing the remnants of the feed and subtracting from the amount of feed (in grams) given to the rats**.**

3.7.3 Determination of body weight gain

The rats in each of the groups were weighed to obtain their initial weights using electronic compact scale (Labtech BL10001). The weight of the rats was monitored on a weekly basis. Body weight gain was determined using the formula;

$$Mean\ Weight\ Gain = \frac{Final\ Bodyweight - Initial\ Bodyweight}{Number\ of\ Animals\ in\ a\ Group} \qquad \textbf{Equation 11}$$

3.7.4 Specific growth rate

Specific growth rate of rats was calculated using the formula**:**

$$\text{Specific growth rate} = \frac{Log(Finalweight) - Log(Initialweight)}{Time} \times 100 \qquad \textbf{Equation 12}$$

3.7.5 Feed efficiency ratio (FER)

The feed efficiency ratio of rats was calculated using the formula:

$$\text{Feed efficiency ratio} = \frac{Bodyweightgain}{Feedintake} \qquad \textbf{Equation 13}$$

3.7.6 Protein efficiency ratio (PER)

Protein efficiency ratio was calculated using the formula:

$$\text{Protein efficiency ratio} = \frac{Bodyweightgain}{Proteinconsumed} \qquad \textbf{Equation 14}$$

3.8 Collection of blood sample

At the end of 28 days feeding experiment the rats were weighed to obtain their final weights. The blood sample was collected according to the method of Shittu *et al*, (2014). Each rat was anaesthetized with chloroform in desiccators and sacrificed. Blood samples were collected in EDTA bottles for haematological analysis and plain bottles for biochemical analysis. Blood collected for biochemical parameters were spun using Eppendorf centrifuge 5702 at 503 x g for 10 minutes to obtain the serum.

3.8.1 Haematological analysis

The following haematological parameters were determined, Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Blood Cell (RBC), Platelets count and Total White Blood Cell differentials (TWBC)using auto hematology analyser (Mindray BC – 5300 model) as described by Akinnawo *et al*. (2002).

3.9 Biochemical Analysis

Serum obtained was analysed for Liver function test and renal function test.

3.9.1 Liver function test

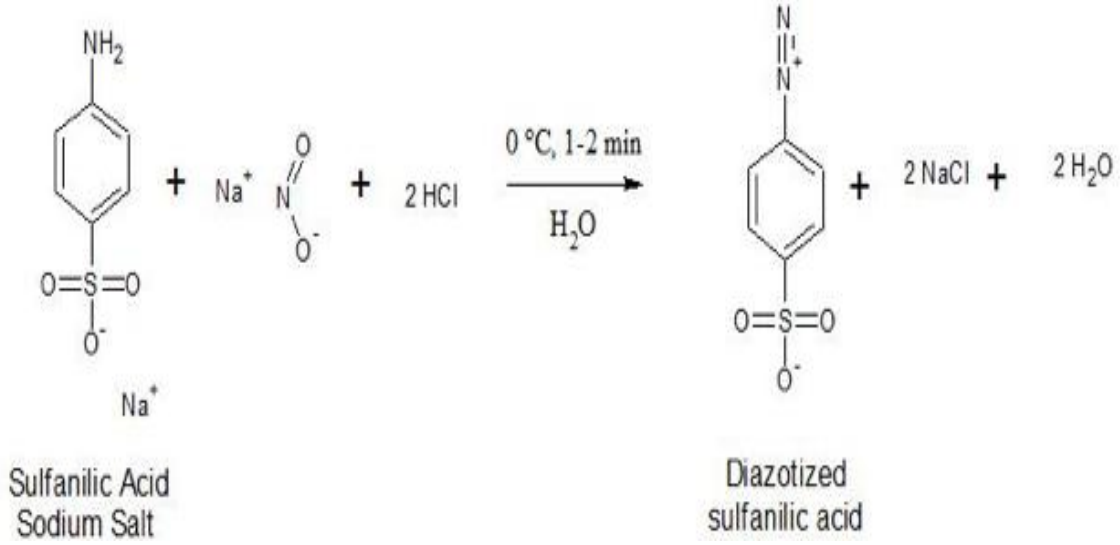
Some serum enzymes were determined for biomarkers of hepatic function using appropriate kits.

3.9.2 Determination of total and conjugated bilirubin

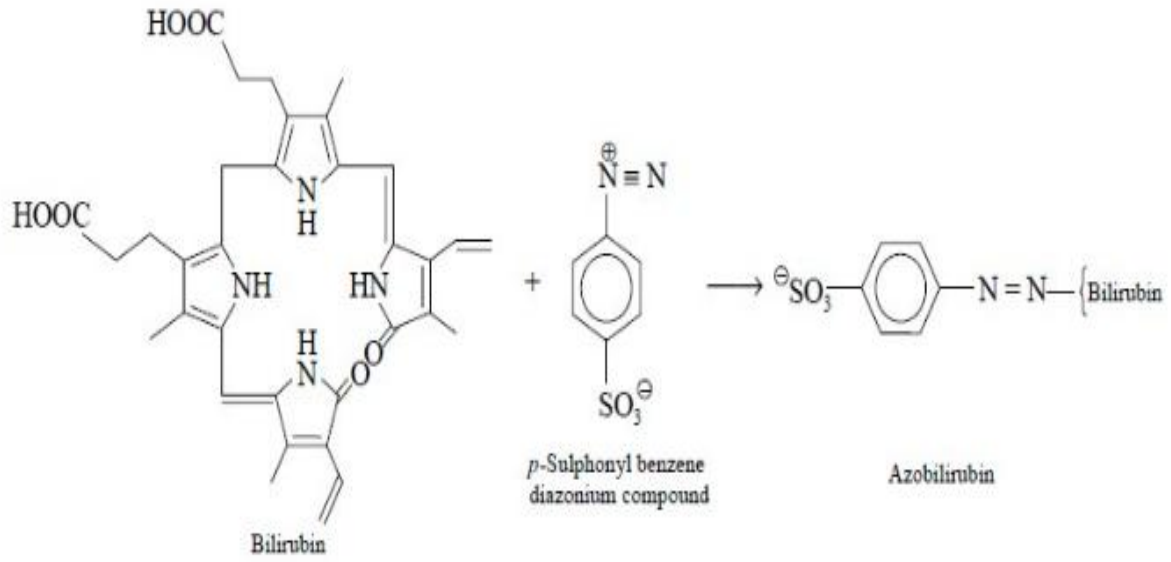
Total and conjugated bilirubin were determined using Diazo method. The principle of Total Bilirubin concentration determination is based on determining the presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely coloured diazo dye (560 nm). The intensity of colour of this dye formed is proportional to the concentration of total bilirubin.

Direct bilirubin is determined in the absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-coloured azobilirubin. The colour intensity of which measured at 546nm is proportional to the intensity of the direct bilirubin in the sample.

Sulfanilic acid + $NaNO_2$ $\xrightarrow{HCl}$ Diazotized sulphanilic acid



Bilirubin + Diazotized sulphanilic acid $\xrightarrow{pH1.4}$ Azobilirubin



Total Bilirubin: 200 µl of sample to be analyzed was introduced into two separate test tubes labeled Sample Blank (^{B}Sample) and Sample (^{A}Sample). Afterwards 200 µl of Sulphanilic acid/HCl and 1.0 µl of caffeine/sodium benzoate was also added followed by the addition of a drop of Sodium Nitrite into ^{A}Sample only.The content of both test tubes were mixed and allowed to incubate in a water bath for 10 minutes at 25 °C. 1.0 ml Tartarate/sodium hydroxide was then added mixed and incubated for 5minutes at 20-25 °Cand absorbance of the sample (^{A}Sample) was read against absorbance of the blank at 578 nm (560- 600 nm) Colour intensity was stable for 30 minutes. Total bilirubin was calculated from the expression;

Total bilirubin (mg/dl) = Sample absorbance X 10.8

Direct Bilirubin: 200 µl of sample to be analyzed was introduced into two separate test tubes labeled Sample Blank (^{B}Sample) and Sample (^{A}Sample). Afterwards 200 µl of Sulphanilic acid/HCl and 2.0 µl of Saline 0.9 % NaCl was also added followed by the addition of a drop of Sodium Nitrite into ^{A}Sample only. The resultant solution in both test

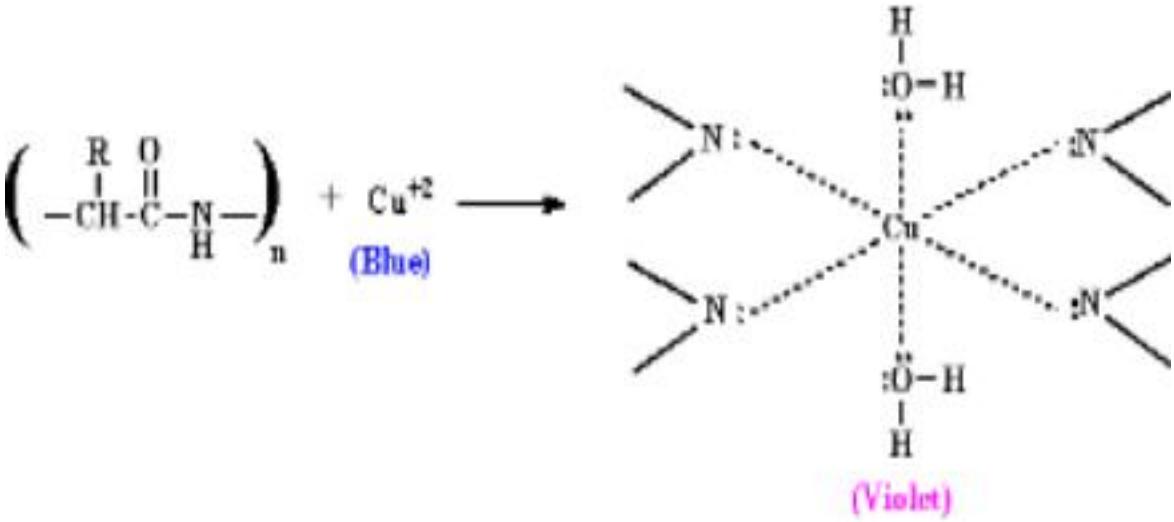
tubes were mixed and allowed to incubate for 5 minutes at 25 °C. The absorbance of the sample (^{A}Sample) was read against absorbance of the blank at 546 nm.

Direct or conjugated bilirubin (mg/dl) = sample absorbance X 14.4

3.9.3 Total protein

Total protein was determined using Spectrum Diagnostic kits.

Principle of protein determination is based on protein peptide bonds react with copper in alkaline medium to form characteristic pink to purple biuret complex. Sodium potassium tartarate prevents copper hydroxide precipitation, and potassium iodide prevents the auto reduction of copper.



Protein concentration is directly proportional to the colour intensity. It is determined by measuring the increase in absorbance at 546nm.

Procedure

Three test tubes were labeled blank, standard and sample and 1.0 ml of Sodium hydroxide/copper sulphate/sodium potassium tartarate/potassium iodine was added to each test tube. This was followed by addition of 20 µl of standard total protein (ST) to the test tube labeled standard and 20 µl of sample to be assayed to the test tube labeled Sample.

The resultant solution was mixed and incubated for 10 minutes at room temperature. The absorbance of sample and standard were measured against the reagent blank within 30 minutes. The total protein concentration in the serum was calculated using equation 15:

Serum protein concentration (g/dl) $= \frac{sampleabsorbanceX\ 6}{standardabsorbance}$ Equation 15

3.9.4 Determination of serum albumin

Albumin concentration was determined using albumin test kit (Spectrum diagnostic kits, Hannover Germany)

Principle

The measurement of serum albumin is based on its quantitative binding to the dye bromocresol green (BCG) at pH 4.1 to form a blue green coloured complex. The intensity of the blue-green colour is directly proportional to the concentration of albumin in the sample. It is determined by monitoring the absorbance at 623 nm.

Albumin + BCG $\xrightarrow{\text{pH 4.1}}$ Albumin –BCG Complex

Procedure

1ml of the reagent Acetate buffer/BCG was transferred into three test tubes labeled blank, standard and specimen.10 µL of standard albumin was added unto the standard and 10 µL of sample added unto the tube labeled specimen. The resultant solution was mixed, incubated for approximately 5 minutes at 20 – 25 ^{o}C and absorbance specimen and standard was read against reagent blank at a wavelength of 623 nm(or 578 nm) within 60 minutes. Albumin concentration was calculated using the formula in equation 16;

$$Concentration\ of\ albumin\ (g/dL) = \frac{Absorbance\ of\ Specimen}{Absorbance\ of\ Standard} \times 4 \quad \textbf{Equation 16}$$

3.9.5 Determination of serum aspertate transaminase (AST) activities

Serum AST was determined using Agappe diagnostic kit.

The principle of AST determination is based on L-Aspartate reacts with α-ketoglutarate in the presence of aspartate amino transferase (AST) to form oxaloacetate + L–Glutamate. The oxaloacetate react with NADH and H^+ in the presence of malate dehydrogenase (MDH) to form L–malate and NAD^+

L–Aspartate + α–ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L–Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{ADH}$ L–malate + NAD^+

Procedure

Exactly 1000 µl of SGOT working reagent was added to 100 µl of sample to be analysed. This was mixed and incubated at 37 ^oC for 1 minute. The change in absorbance per minute (OD/min) measured within 3 minutes.

SGOT (AST) activity was calculated from the expression below:

SGOT (AST) (U/L)= (ΔOD/mins) X 1745.

Where: ΔOD= change in absorbance or optical density

1745= the provided factor for estimation of assay (Normal procedure & High linearity procedure) in semi auto analyser.

3.9.6 Determination of serum alkaline phosphatase activities (ALP)

Alkaline phosphates (ALP) was determined using Agappe diagnostic kits. The principle of ALP determination is based on the para-nitrophenyl phosphate reacts with water in the presence of alkaline phosphatase to produce para-nitrophenol + inorganic phosphate.

P–nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ P – nitrophenol + inorganic phosphate

Procedure

Exactly 1000 µl of ALP working reagent was added to 20 µl of sample to be analysed. This was mixed and incubated at 37 ^{o}C for one minute. The change in absorbance per minute (ΔOD/min) was measured within 3 minutes.

The alkaline phosphatase activity was calculated using the formula below;

Alkaline phosphatase activity (u/l) = (ΔOD/min) X 2750

Where; 2750 = the provided factor for estimation of ALP using Semi Auto Analyser

ΔOD= change in absorbance or optical density.

3.9.6 Determination of serum AlanineTransaminaseALT) activities

Serum Alanine Transaminase (ALT) activity was determined using Agappe diagnostic kits.

Principle

Alanine Transaminase catalyses the reaction involving the transamination of alanine and α – oxoglutarate to form pyruvate and glutamate. The activity of ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, - dinitrophenylhydrazine.

α – oxoglutarate + alanine $\xrightarrow{\text{ALT}}$ L-glutamate + Pyruvate

Procedure

Exactly 1000 µl of ALT working reagent was added to 100 µl of sample to be assayed. The resultant solution was mixed and allowed to incubate at 37 ^{o}C for 1 minute. The change in absorbance per minute (ΔOD/min) was measured over a period of 3 minutes.

The ALT Activity was calculated using the formula,

ALT activity (U/L) = (ΔOD/min) x 1745

3.10 Renal Function Test

Some serum enzymes were determined for biomarkers of renal function using Spectrum & Teco diagnostic kits.

3.10.1 Determination of serum urea concentration

Urea was determined using Spectrum Assay colorimetric method

Principle

The principle is based on the hydrolysis of urea in the presence of water and urease to produce ammonia and carbon dioxide according to the reaction below:

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

The free ammonia in an alkaline pH and in the presence of dye forms coloured complex proportional to the urea concentration in the specimen.

Procedure

Three (3) test tubes were labeled blank, standard and specimen. To each of the test tube, 1.0 ml of reagent containing phosphate buffer pH 8.0, Sodium Salicyclate, sodium nitroprisside and EDTA was added followed by 50 µl (one drop) of urease enzyme. Approximated 10 µl of standard urea was added to only the test tube labeled standard and 10 µl of sample to be analysed was added to the test tube labeled sample. The resulting solutions were mixed and incubated for 10 mins at 25 ^oC and absorbance of standard and sample measured against reagent blank. The serum urea concentration was calculated using the formula;

$$\text{Serum Urea Concentration (mg/dl)} = \frac{Absorbance of the sample X\ n}{Absorbance of the standard} \qquad \textbf{Equation 17}$$

Where n =50.0 mg/dl (8.33 mmol/l)

Urine urea concentration is determined by multiplying the result by the dilution factor (50).

3.10.2 Serum creatinine concentration

The creatinine concentration was determined using spectrum diagnostic kit.

Principle

The principle is based on the reaction of creatinine with picric acid under alkaline condition to form a yellow-red complex. The absorbance of the coloured product was measured at a wavelength 492 nm. The observed value is directly proportional to creatinine concentration in the sample.

Creatinine + Picrate $\xrightarrow{\text{Alkaline pH}}$ yellowed complex

Procedure

A 1.0 ml of working solution (consisting of one volume picric acid + one volume sodium hydroxide) was added unto test tubes labeled standard and sample. 100 µl of standard solution was added to the test tubes labeled standard and 100 µl of sample to be analysed added to the test tube labeled sample. The resulting solution was mixed and after 30 seconds, the first absorbance (A1) of both standard and sample was read and 2 minutes later, the second absorbance (A2) of standard and sample read. The serum creatinine concentration was calculated from the formula below;

Serum creatinine concentration (mg/dl) $= \frac{Absorbance of the sample X\ 2}{Absorbance of the standard}$ **Equation 18**

Urine creatinine Concentration (mg/dl): $= \frac{Absorbance of the sample X\ 2\ X\ 50}{Absorbance of the standard}$ **Equation 19**

3.10.3 Serum potassium concentration

Potassium concentration was determined using Teco Diagnostic Kits. The principle is based on the amount of potassium is determined by using sodium tetraphenylboron in a

specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2- 7 mEq/L.

Procedure

Three (3) test tubes were labeled standard, sample and blank. A 1.0 µl of potassium reagent was pipette into all the test tubes labeled sample, standard and blank.this was followed by addition of 0.01 ml (10 µl) of samples to test tubes labeled standard and sample and distilled water to blank. The resulting solutions were mixed and left standing at room temperature for 3 minutes. The absorbencies of sample and standard were read at 500 nm after which the spectrophotometer was zeroed in each case with the blank before taking another reading. Potassium concentration (mEq/L) was calculated using the formula.

$$\text{Potassium conc. (mEq/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of } standard} \text{ X conc. of standard (mEq/L)}$$

Equation 20

3.10.4 Serum Sodium concentration

Serum sodium was determined using Teco Diagnostics Kit. The present method is based on modification of those first described by maruna and Trinder in which sodium is precipitated as the triple salt, sodium magnesium Uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Procedure

Three (3) test tubes were labeled standard, sample and blank. A 1.0 ml of filtrate reagent (Uranyl acetate 2.1 mM and Magnessium Accetate 20 mM in ethyl alcohol) followed by 50 µl of sample to all tubes and distilled water to the blank. The resulting solutions were mixed by shaking vigorously and continuously for 3 minutes. They were then centrifuged at

high speed 1,500 g for 10 minutes. The resulting supernatant fluids were then collected. Corresponding test tubes to the above filtrate tubes were also labeled after which 1.0 ml of acid reagent (diluted acetic acid) was pipette into all the test tubes followed by 50 µl of supernatant and 50 µl of colour reagent to all test tubes and mixed. The absorbance of all the test tubes was read at 550 nm and sodium concentration calculated from the formula:

$$Conc.\ of\ Sodium\ (mEq/L) = \frac{Abs\ of\ blank - Abs\ of\ Sample}{Abs\ of\ blank - Abs\ of\ Standard} \times Conc.\ of\ Standard$$

***Equation* 21**

3.10.5 Determination of serum chloride concentration

Chloride was determined using spectrum diagnostic kits

The principle is based on the chloride ions react with Mercucury (Hg) II thiocyanate in acidic medium to form thiocyanate. These ions further react with HNO_3 and FeIII-ions and effect a red colour. The increased extinction (coloration) is directly proportional to the chloride ions concentration.

Procedure

One ml (1.0 ml) of reagent (R) (containing 2 mmol/l Hg II-thiocyanate, 30 mmol/l Fe III-Nitrate, 40 mmol/l HNO_3) was pipette into clean test tubes labeled blank, standard and sample. Exactly 10 µl of the standard (S) (containing 100 mmol/l (354.6 mg/dl) chloride) was added to the test tube labeled standardwhile 10µl of sample to be analysed was added to the test tube labeled sample. The resulting content was mixed well and left to stand for 5 minutes. The absorbance of the standard and samplewas read against the reagent blank at 492 nm. Chloride concentration in the serum was calculated using the equation 22:

Serum chloride concentration (mmol/L) $= \frac{\Delta A\ of the sample X\ 100}{\Delta A of the standard}$ **Equation 22**

Where: ΔA = Change in Absorbance

3.11 Histopathological study of organs

Three different tissues, liver, kidney and duodenum were processed for histopathological studies.

Principle

Alum acts as mordant and haematoxylin containing alum stains the nucleus light blue. This is achieved through bluing process which converts the initial soluble red colour within the nucleus to an insoluble blue colour.The counterstaining is done by using eosin which imparts pink colour to the cytoplasm (Avwioro, 2002).

Procedure

Sections from each of the harvested organs (liver, kidney and heart) were carried out in the histopathology laboratory of the University of Ilorin Teaching Hospital, Ilorin Kwara state.

Fixation: the tissue were fixed in 10 % formalin for 48 hours.

Grossing: the organs were gross into smaller pieces about a size that will fit into the cassette.

Tissue processing: Group A-F were hydrated, processed using ascending grade of alcohol from 10 % -20 % -30 % -40 % -50 % -60 % -70 % -80 % -90 % -100 % (absolute) for 1 hour for each changes.

Cleaning: All tissue labeled group A-F were processed using clearing agent opf xylene. This is to remove alcohol from the tissue for 45 minutes.

Infiltration: The clear tissues were infiltrated in two changes of molten paraffin wax 1 hour in each change.

Embedding: The tissue were embedded in molten paraffin wax using disposable cassette and allowed to solidify before microtomy.

Microtome: Tissue block were all sectioned at 4µm using rotary microtome, section were floated in warm water bath and each picked in pairs on albumized glass slide.

Staining using Hematoxylin & Eosin (H&E): slide were dewaxed in xylene for 15 minutes then stained in Haematoxylin solution for 10 minutes and afterwards rinsed in water. It was then blue in running tap water for 10 minutes and counterstained in 1 % eosin solution for 1 minute then rinsed in water and finally mounted in Dibutylphthalate polystyrene xylene (DPX) for micrioscopy.Organ pathological changes were scored in the rats according to Fadina *et al*. (1999). Photomicrographs of some of the lesions were taken using a Microscope fitted with a Leitz camera unit, and processed routinely in a colour photo laboratory.

3.12 Data analysis

All data were in triplicate. Statistical analysis was performed using one way analysis of variance (ANOVA), Duncan multiple range test (DMRT) using the computer software Statistical Package for Social sciences (SPSS) version 2.2.0. Data were analyzed at a 95 % confidence interval. Significance differences among means were determined at $p < 0.05$ and values were expressed as mean ± SEM.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Result

The proximate composition of *Cirina forda* (optimised and non-optimised), cassava flakes and soybean are as shown in Table 4.1. The highest moisture content was found in Cassava flakes (13.36 %) while soybean had the least. There was no significant difference (P>0.05) in the moisture content of optimised *Cirina forda* and non-optimised *Cirina forda*. Optimized *Cirina forda* was found to contain the the highest protein but not significantly higher (P>0.05) than in the non-optimised. Optimization only increased the protein by approximately 5 %. Soybean was found to contain 40 % protein while cassava flakes had 3.8 %. Fat content for soybean was found to be much higher than that of cassava flakes while the *Cirina forda* (optimized and non-optimized) were found to be similar (9.88±0.07 % and 9.01±0.12 % respectively). Cassava flakes had the highest NFE value (77.19 ± 0.52 %) while optimized *Cirina forda* had the lowest 11.44±1.14 %. Crude fibre content ranged from 1.51±0.10 % in cassava flakesto 8.40±0.02 % in non-optimised *Cirina forda*. There was no significant difference (P>0.05) in the ash content of all the diets with exception of the basal diet which was found to contain 1.34±0.05 % of ash.

Table 4.1: Proximate composition of optimised and non-optimised *Cirina forda*, cassava flakes and soybean (%)

Parameters	Cassava Flakes	Optimised *Cirina forda*	Non-optimised *Cirina forda*	Soybean
Moisture	13.360±0.450^{d}	7.425±0.085^{b}	8.825±0.135^{c}	4.490±0.120^{a}
Crude protein	3.805±0.105^{a}	55.345±0.345^{d}	51.335±0.425^{c}	40.220±0.210^{b}
Fat	2.805±0.105^{a}	9.880±0.070^{c}	9.010±0.120^{b}	20.315±0.025^{d}
NFE	77.190±0.520^{d}	11.440±1.140^{a}	15.900±0.710^{b}	22.905±0.265^{c}
Crude fibre	1.505±0.095^{a}	8.030±0.030^{c}	8.400±0.020^{d}	5.185±0.105^{b}
Ash	1.335±0.045^{a}	7.880±0.980^{b}	6.530±0.050^{b}	6.980±0.080^{b}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

The result in Table 4.2 shows the anti-nutrient composition of cassava flakes, soybean, optimised and non-optimised *Cirina forda.* Cyanogenic glycoside was generally low and ranged between 0.04±0.01 mg/100g in soybean to 3.923±0.16 mg/100g in cassava flakes. Saponins were generally high in non-optimised and optimized *Cirina forda* (362.023±1.291 mg/100g; 231.390±2.350 mg/100g respectively) followed by tannins (183.423±1.51 mg/100g;116.77±1.29 mg/100g) and alkaloids (62.09±0.81; 66.74±0.98 mg/100g). This same trend was also observedin cassava flakes and soybean. Cassava flakes had the highest concentration of oxalate (13.75±0.77 mg/100g) while soybean had the least concentration (2.25±0.10 mg/100g). There was no significant difference (P>0.05) in the oxalate concentration of optimized and non optimized insect samples. The phytate concentration of optimized and non-optimised were not significantly different (P>0.05) and were lower than those of cassava fakes and soybeans.

Table 4.2: Anti-nutrient composition of optimised and non-optimised *Cirina forda*, cassava flakes and soybean (mg/100g)

Minerals	Non-optimized *Cirina forda*	Optimized *Cirina forda*	Cassava Flakes	Soybean
Cyanogenic Glycocides	3.513±0.297[b]	2.363±0.166[b]	3.923±0.160[c]	0.040±0.010[a]
Phytate	16.747±0.786[a]	17.073±1.182[a]	29.047±1.250[b]	210.707±1.462[c]
Alkaloids	62.093±0.807[b]	66.740±0.981[c]	52.680±1.060[a]	323.737±2.887[d]
Saponins	362.023±1.291[c]	231.390±2.350[b]	32.480±01.486[a]	402.750±2.356[d]
Oxalate	2.520±0.000[b]	2.790±0.140[c]	13.750±0.771[d]	2.253±0.103[a]
Tannins	183.423±1.507[d]	116.773±1.293[c]	21.737±0.613[a]	27.803±0.918[b]

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

The mineral composition of optimised and non-optimised *Cirina forda*, cassava flakes and soybean are shown in Table 4.3. There was no significant difference ($P>0.05$) in the concentration of Na, Zn and Fe in non-optimised and optimized insect samples. The concentration of Mn, Ca, Cr, Cu, and P was significantly higher ($P<0.05$) in non-optimised *Cirina forda* when compared to the optimized *Cirina forda*. The most abundant mineral was K for cassava flakes and soybean and phosohorus for optimized and non-optimised *cirina forda*. Howevertheleast abundant mineral in all the groups was chromium (Cr).

Table 4.3: Mineral composition of optimised and non-optimised *Cirina forda,* cassava flakes and soybean

Mineral	Non-optimized *Cirina forda*	Optimized *Cirina forda*	Cassava flakes	Soybean
Sodium (Na)	0.866±0.001[b]	0.914±0.050[b]	0.558±0.001[a]	8.310±0.000[c]
Manganese (Mn)	0.047±0.000[b]	0.035±0.000[a]	0.091±0.000[c]	2.870±0.001[d]
Zinc (Zn)	0.138±0.000[a]	0.136±0.000[a]	0.141±0.001[a]	2.505±0.005[b]
Potassium (K)	0.967±0.000[a]	1.018±0.000[b]	5.102±0.002[c]	793.010±0.010[d]
Calcium (Ca)	1.595±0.005[c]	0.711±0.000[a]	0.952±0.003[b]	281.535±0.005[d]
Iron (Fe)	0.075±0.001[a]	0.075±0.000[a]	0.122±0.001[b]	12.385±0.005[c]
Chromium (Cr)	0.004±0.000[a]	0.002±0.000[a]	0.079±0.003[c]	0.041±0.001[b]
Copper (Cu)	0.032±0.000[b]	0.005±0.000[a]	0.079±0.002[c]	1.931±0.001[d]
Magnesium (Mg)	0.942±0.000[a]	1.026±0.001[b]	1.484±0.004[c]	264.325±0.005[d]
Phosphorus (P)	3.456±0.001[c]	2.349±0.000[b]	2.115±0.006[a]	684.005±0.005[d]

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

The result in Table 4.4 shows that the non optimized *Cirina forda* had the lowest total amino acid composition while soybean had a significantly higher value ($P<0.05$). The Non essential Amino Acid (NEAA) composition in the samples is as shown in Table 4.6. The non-optimised *Cirina forda* was significantly higher ($P<0.05$) in the composition of the total non-essential amino acid compared to optimized *Cirina forda* and soybean except for the concentration of serine which was not significant ($P>0.05$) for all the samples. The higher values of NEAA in non-optimised *Cirina forda* over the optimized and soybean was not significant ($P<0.05$) for serine. Alanine content of non-optimised *Cirina forda* was significantly higher ($P<0.05$) than optimized *Cirina forda* and soybean.

Optimised *Cirina forda* had a significantly higher ($P<0.05$) total sulphur containing amino acids, total non-sulphur containing amino acids, total non-aromatic amino acids and total basic amino acids compared with non-optimised *Cirina forda* as shown in Table 4.7 but significantly lower ($P<0.05$) when compared with the reference protein soybean. Total aromatic amino acids was significantly higher ($p<0.05$) in non-optimised *Cirina forda* compared with optimized *Cirina forda* but also significantly lower ($P<0.05$) when compared with soybean.

The essential amino acids composition is as shown in Table 4.5. There was no significant difference ($P>0.05$) in the concentration of Phenylalanine, Tryptophan and Histidine in optimized *Cirina forda* when compared with the non-optimized *Cirina forda*. The concentration of the other essential amino acids (Leucine, Lysine, Isoleucine, Valine, Methionine and Arginine) were significantly higher ($P<0.05$) in optimized *Cirina forda* compared to non-optimised *Cirina forda* but significantly lower ($P>0.05$) compared to the reference protein soybean except for leucine and arginine.

Table 4.4: Amino acid composition in *Cirina forda* (optimised and non-optimised) and soybean (g/100g)

Parameters	Optimised *Cirina forda*	Non-optimised *Cirina forda*	Soybean
Leucine*	8.230±0.060^{b}	7.790±0.030^{a}	7.730±0.030^{a}
Lysine*	4.585±0.025^{b}	4.215±0.025^{a}	6.680±0.000^{c}
Isoleucine*	4.060±0.070^{b}	3.535±0.065^{a}	5.465±0.035^{c}
Phenylalanine*	3.635±0.085^{a}	3.635±0.085^{a}	5.095±0.045^{b}
Tryptophan*	0.920±0.030^{a}	0.815±0.025^{a}	1.445±0.025^{b}
Valine*	5.085±0.055^{b}	4.355±0.085^{a}	5.700±0.030^{c}
Methionine*	1.685±0.025^{b}	1.415±0.025^{a}	1.630±0.030^{b}
Proline	4.060±0.000^{b}	3.755±0.105^{ab}	3.550±0.100^{a}
Arginine*	7.785±0.045^{b}	7.225±0.005^{a}	7.140±0.090^{a}
Tyrosine	3.525±0.085^{a}	4.385±0.085^{b}	3.695±0.085^{a}
Histidine*	1.980±0.000^{a}	1.720±0.000^{a}	2.680±0.000^{a}
Cystine	1.450±0.000^{a}	1.450±0.120^{a}	1.880±0.060^{b}
Alanine	3.755±0.035^{a}	5.045±0.035^{b}	4.740±0.110^{b}
Glutamic acid	14.765±0.075^{b}	16.125±0.075^{c}	11.735±0.075^{a}
Glycine	4.040±0.190^{a}	3.585±0.025^{a}	4.750±0.050^{b}
Threonine*	4.465±0.085^{a}	4.245±0.085^{a}	4.270±0.110^{a}
Serine	3.700±0.030^{a}	3.535±0.025^{a}	3.615±0.055^{a}
Aspartic acid	10.390±0.030^{b}	9.860±0.060^{a}	9.800±0.060^{a}
∑ amino acids	**88.12**	**86.69**	**91.60**

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at (P≤0.05).* Essential Amino Acids

Table 4.5: Essential amino acids composition in *Cirina forda* (optimised and non-optimised) and soybean (g/100g)

Parameters (g/100g)	Optimised *Cirina forda*	Non-optimised *Cirina forda*	Soybean
Leucine	8.230±0.060^{b}	7.790±0.030^{a}	7.730±0.030^{a}
Lysine	4.585±0.025^{b}	4.215±0.025^{a}	6.680±0.000^{c}
Isoleucine	4.060±0.070^{b}	3.535±0.065^{a}	5.465±0.035^{c}
Phenylalanine	3.635±0.085^{a}	3.635±0.085^{a}	5.095±0.045^{b}
Tryptophan	0.920±0.030^{a}	0.815±0.025^{a}	1.445±0.025^{b}
Valine	5.085±0.055^{b}	4.355±0.085^{a}	5.700±0.030^{c}
Methionine	1.685±0.025^{b}	1.415±0.025^{a}	1.630±0.030^{b}
Arginine	7.785±0.045^{b}	7.225±0.005^{a}	7.140±0.090^{a}
Histidine	1.980±0.000^{a}	1.720±0.000^{a}	2.680±0.000^{a}
Threonine	4.465±0.085^{a}	4.245±0.085^{a}	4.270±0.110^{a}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

Table 4.6: Non-essential amino acids composition in *Cirina forda* (optimised and non-optimised) and soybean (g/100g)

Parameters	Optimised *Cirina forda*	Non-optimised *Cirina forda*	Soybean
Proline	4.060±0.000^{b}	3.755±0.105^{ab}	3.550±0.100^{a}
Tyrosine	3.525±0.085^{a}	4.385±0.085^{b}	3.695±0.085^{a}
Cystine	1.450±0.000^{a}	1.450±0.120^{a}	1.880±0.060^{b}
Alanine	3.755±0.035^{a}	5.045±0.035^{b}	4.740±0.110^{b}
Glutamic acid	14.765±0.075^{b}	16.125±0.075^{c}	11.735±0.075^{a}
Glycine	4.040±0.190^{a}	3.585±0.025^{a}	4.750±0.050^{b}
Serine	3.700±0.030^{a}	3.535±0.025^{a}	3.615±0.055^{a}
Aspartic acid	10.390±0.030^{b}	9.860±0.060^{a}	9.800±0.060^{a}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

Table 4.7: Classes of amino acids composition in *Cirina forda* (optimised and non-optimised) and soybean

Class of Amino Acid	Optimised *Cirina forda*	Non-optimised *Cirina forda*	Soybean
TAA	88.120±0.010[b]	86.690±0.001[a]	91.600±0.001[c]
TEAA:TNEAA	0.930±0.001[b]	0.821±0.001[a]	1.090±0.001[c]
TSAA:TNSAA	0.041±0.001[b]	0.031±0.001[a]	0.041±0.001[b]
TArAA:TNArAA	0.091±0.001[a]	0.101±0.001[b]	0.110±0.001[c]
TEAA with Histidine	42.430±0.001[b]	38.950±0.001[a]	47.840±0.001[c]
TEAA without Histidine	40.450±0.001[b]	37.230±0.001[a]	45.161±0.001[c]
TNEAA	45.690±0.001[b]	47.740±0.000[c]	43.770±0.001[a]
TSAA	3.140±0.001[b]	2.870±0.000[a]	3.515±0.005[c]
TNSAA	84.985±0.005[b]	83.830±0.001[a]	88.095±0.005[c]
TArAA	7.165±0.005[a]	8.025±0.005[b]	8.795±0.004[c]
TNArAA	80.915±0.005[b]	78.675±0.005[a]	82.815±0.005[c]
TAAA	25.180±0.020[b]	25.989±0.001[c]	21.540±0.001[a]
TBAA	14.360±0.010[b]	13.165±0.005[a]	16.505±0.005[c]

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at (P≤0.05).

TAA	Total Amino Acids
TEAA:TNEAA	Total Essential Amino Acids : Total Non Essential Amino Acids
TSAA: TNSAA	Total Sulphur Amino Acids: Total Non Essential Amino Acids
TArAA: TNArAA	Total Aromatic Amino Acids: Total Non Aromatic Amino Acids
TEAA (With Histidine)	Total Essential Amino Acids with Histidine
TEAA(WithoutHistidine)	Total Essential Amino Acids without Histidine
TNEAA	Total Non Essential Amino Acids
TSAA	Total Sulphur Amino Acids
TNSAA	Total Non Sulphur Amino Acids
TArAA	Total Aromatic Amino Acids
TNArAA	Total Non Aromatic Amino Acids
TAAA	Total Acidic Amino Acids
TBAA	Total Basic Amino Acids

The result in Figure Figure 4.1. Shows the feed intake of group of rats placed on the various supplemented diets. Significant decrease ($P<0.05$) in the feed intake of all the animals was observed in the first week of the experimentalthough not significant in the animals placed on 20 % soybean diet. The feed intake increased gradually in all the groups to the end of the experiment (week 3). Animals placed on 20 % soyabean protein had significantly higher ($P<0.05$) feed intake while those fed 20 % non-optimised *Cirina forda* had significantly lower ($P<0.05$) feed intake when compared with the other groups.

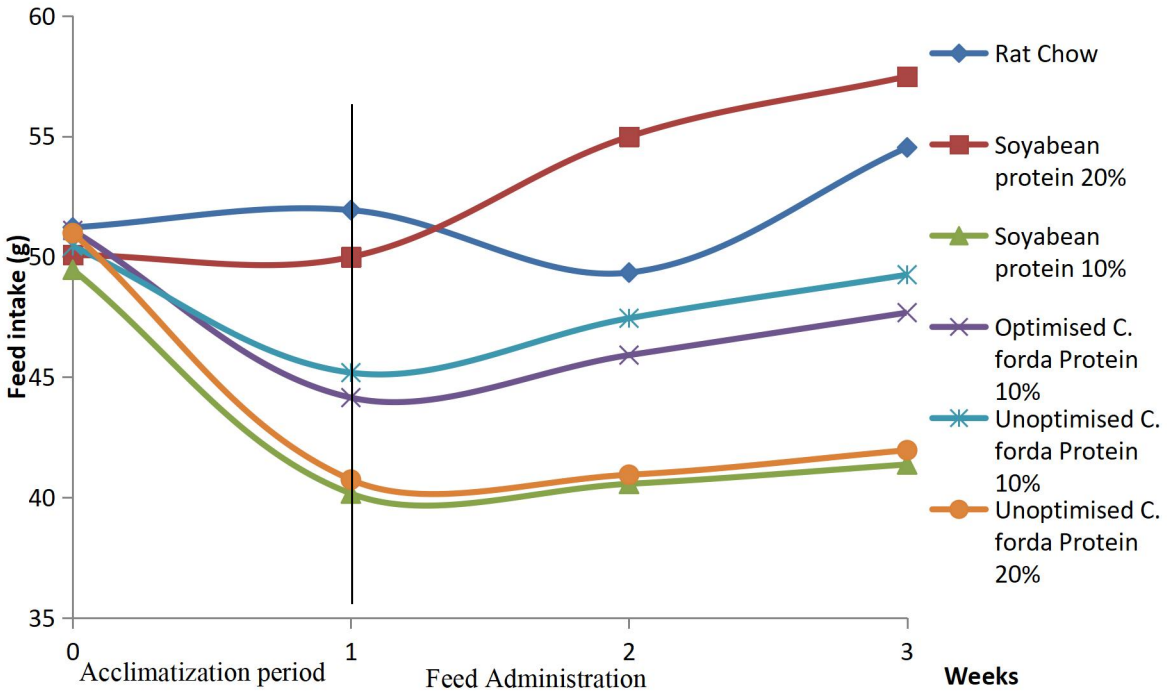


Figure 4.1: Weekly feed intake of rats fed with Soybean protein, Optimised and Non-Optimised *Cirina forda* protein supplemented diets.

The weekly weight gain of rats fed with different supplemented diets is as shown in Figure 4.2. Rats fed with 20 % non-optimised *Cirina forda* experienced a significant weight loss after week 1 of experiment but picked up gradually in week 2. The weight gain of rats fed 20 % soybean protein was progressive and singnificantly higher ($P<0.05$) than those placed on other supplemented diets. There was no significant ($P>0.05$) weight gain in the other groups fed with 10 % soybean, 10 % optimized *Cirina forda*, rat chow (control) and 10 % non-optimised *Cirina forda* supplemented diet.

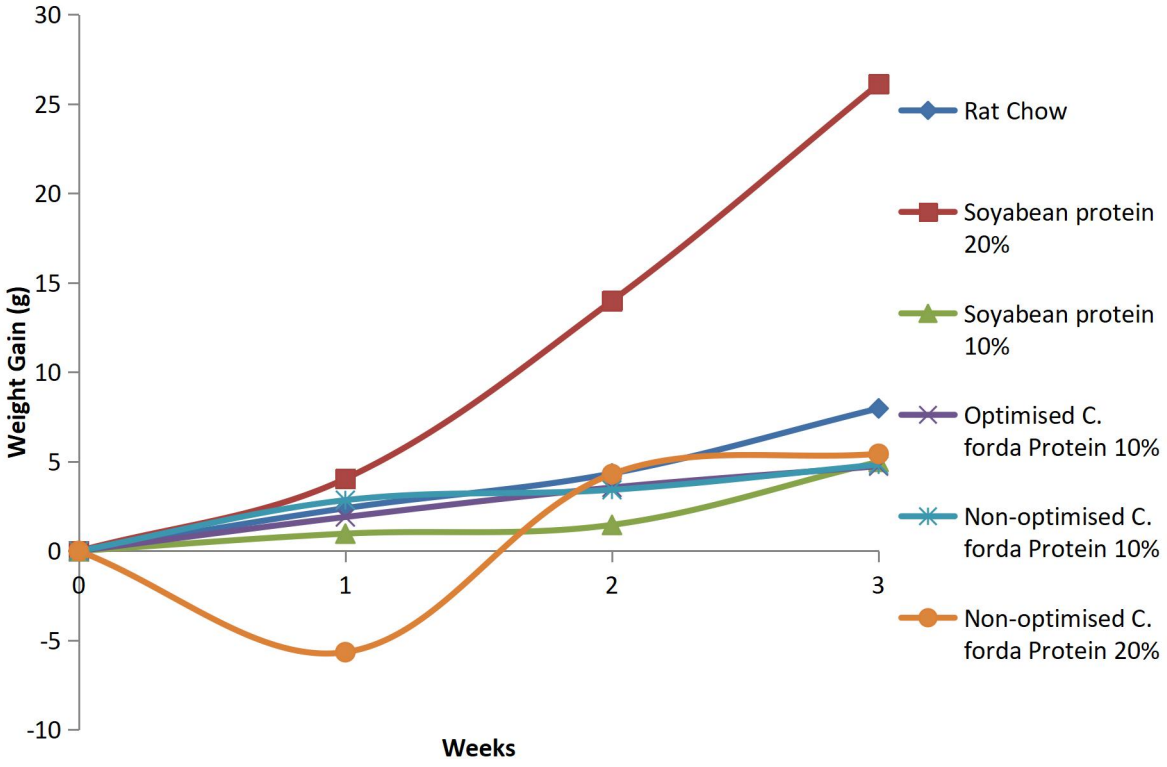


Figure 4.2: Weekly weight gain of rats fed with soybean protein, optimised and non-optimised *Cirina forda* protein supplemented diets

The results in Table 4.8 shows the hematological parameters of rats fed with different supplemented diets. A significant difference ($P<0.05$) was observed for all the hematological parameters studied for the various animal groups. The group fed with 20 % soybean protein had the highest heamoglobin level (14.75±0.104g/dL) while the group fed 10 % non-optimised *Cirina forda* had the lowest hemoglobin level (9.53±0.301 g/dL) compared to other groups. Similar trend was observed for PCV and MCV.

There was no significant difference ($P\leq0.05$) in MCHC among the groups fed with different supplemented diets. The highest MCHC value was found in groups fed with control diet and 10 % Soybean protein (33.250 g/dL) while the least value was recorded in groups fed 10 % optimised *Cirina forda* protein (32.00 g/dL).

The RBC level was significantly higher ($P<0.05$) for the group fed with 20 % Soybean protein (5.03 g/dL) and significantly lower for the group fed with 10 % Soybean protein (4.13 g/dL), while insects supplemented diets were not significantly different ($P>0.05$) from control diet (rat chow).

The platelet level was highest in the group of animal fed with 10 % non-optimised *Cirina forda* protein (313.75) while the least platelet level was observed in groups fed with 20 % non-optimised *Cirina forda*.

The highest concentration of total white blood cells (TWBC) was observed in groups fed with *control diet* (10.63) which was not significantly different ($P>0.05$) from the other groups.

Table 4.8: Hematological Parameters of Rats Fed with *Cirina forda* (optimized and non-optimised) and soybean diet supplements

Parameters	Control diet	10 % Soybean	20 % Soybean	10 % Optimised *Cirina forda*	10 %Non-optimised *Cirina forda*	20 % Non-optimised *Cirina forda*
Hb (g/dL)	11.775±0.338^{c}	11.300±0.358^{c}	14.750±0.104^{e}	10.450±0.272^{b}	9.525±0.301^{a}	13.150±0.144^{d}
PCV (%)	35.000±1.080^{c}	33.250±1.109^{bc}	44.000±0.408^{e}	31.250±0.853^{b}	28.250±0.853^{a}	39.000±0.408^{d}
MCV (FI)	74.500±2.723^{bc}	80.750±5.072^{bcd}	88.750±1.109^{d}	71.750±3.326^{b}	60.250±1.931^{a}	82.000±2.041^{cd}
MCH (Pg)	24.500±0.957^{b}	27.500±1.756^{bc}	29.500±0.289^{c}	25.000±2.041^{b}	20.000±0.707^{a}	27.000±0.577^{bc}
MCHC (g/dL)	33.250±0.250^{a}	33.250±0.479^{a}	33.000±0.000^{a}	32.000±1.000^{a}	33.000±0.000^{a}	32.750±0.250^{a}
RBC	4.700±0.122^{ab}	4.125±0.149^{a}	5.025±0.103^{b}	4.450±0.343^{ab}	4.525±0.272^{ab}	4.750±0.104^{ab}
PLT	282.000±6.583^{ab}	275.000±12.865^{ab}	258.000±10.198^{a}	268.000±7.012^{a}	313.750±27.672^{b}	247.500±6.436^{a}
TWBC	10.625±0.312^{b}	9.650±0.463^{ab}	9.850±0.132^{b}	8.175±0.666^{a}	10.200±0.738^{b}	9.625±0.405^{ab}

Values are mean ± standard errorof triplicate determinations, values on the same row with different superscripts are significant at ($P \leq 0.05$).Hb=Haemoglobin, PCV=Packed cell volume, MCV=Mean corpsular Volume,MCH= Mean corpuscular Haemoglobin, MCHC=Mean corpuscular Haemoglobin concentration,RBC=Red blood cell, PLT=Platelet, TWBC=Total white blood cell.

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The liver function parameters of rats fed on various supplemented diets are presented in Table 4.9 Aspartate transaminase (AST) activity was significantly higher ($p<0.05$) in the group fed 20 % non-optimised *Cirina forda* whencompared with the other groups. The activity of AST was lowest in the control group.

Alanine transaminase (ALT) in the liver of rats fed 20 % non-optimised *Cirina forda* and 10 % non-optimised *Cirina forda* were significantly higher ($P<0.05$) than in the optimized, soybean and control groups.

Alkaline phosphate (ALP) activity was significantly different among the different groups. It was found to be highest in the group fed with 20 % non-optimised *Cirina forda* and lowest in the group fed with 20 % soybean diet.

Total protein (TP) concentration was highest in the group fed 20 % soybean and lowest in group fed 20 % non-optimised *Cirina forda* protein and was significantly different from the other groups.

The highest Albumin concentration was observed in the group fed 10 % non-optimized C*irina forda* and lowest in the group fed 20 % non-optimized C*irina forda*. The albumin concentration of the groups fed 10 % optimized and 10 % non optimized C*irina forda* was not significant ($P>0.05$) when compared with the group fed the control diet (rat chow).

The group of rats fed 20 % non-optimized *Cirina forda* had a significantly higher ($P<0.05$) total bilirubin (B1) and Conjugated bilirubin (B2) while the control group had the significantly lower ($P<0.05$) total bilirubin.

Table 4.9: Liver function parameters of rats fed with soybean, optimized and non-optimised *Cirina forda* supplemented diets

Parameters	Rat chow (Control diet)	10 % Soybean	20 % Soybeans	10 % optimised	10 % non-optimised *Cirina forda*	20 % non-optimised *Cirina forda*
AST	26.570±0.010^{b}	26.265±0.005^{a}	28.365±0.005^{d}	27.060±0.060^{c}	39.730±0.010^{e}	48.085±0.015^{f}
ALT	29.355±0.005^{a}	33.195±0.015^{b}	37.330±0.010^{d}	34.585±0.005^{c}	61.635±0.005^{e}	78.750±0.010^{f}
ALP	128.370±0.010^{c}	117.450±0.010^{b}	114.570±0.010^{a}	143.385±0.005^{d}	157.580±0.020^{e}	215.745±0.005^{f}
TP	6.050±0.050^{b}	6.415±0.015^{c}	7.100±0.100^{d}	6.220±0.020^{b}	6.210±0.020^{b}	3.050±0.050^{a}
ALB	3.050±0.050^{d}	2.895±0.005^{c}	2.705±0.005^{b}	3.050±0.005^{d}	3.150±0.050^{d}	2.050±0.050^{a}
TB	9.050±0.050^{a}	17.050±0.050^{b}	19.100±0.100^{c}	17.050±0.050^{b}	22.050±0.050^{d}	38.150±0.150^{e}
CB	4.045±0.045^{a}	6.995±0.005^{b}	9.100±0.100^{c}	7.050±0.050^{b}	11.045±0.045^{d}	15.100±0.100^{e}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).AST= Aspatate Transaminase ALT= Alanine Transaminase, ALP = Alkaline phosphatase, TP=Total protein, ALB= Albumin, TB=Total Bilirubin, CB= Conjugated Bilirubin

The renal function parameters of rats fed with the different supplemented diets are presented in Table 4.10. The urea concentration of group of rats fed 20 % non-optimised *Cirina forda*, 20 % soybeanand 10 % optimized *Cirina forda* were significantly higher ($P<0.05$) than the urea concentration of all the other groups. The highest creatine concentration was observed in the group fed 20 % non-optimised *Cirina forda* and lowest in the group fed rat chow (control). No significant difference ($P>0.05$) was observed in the potassium concentration of groups fed 10 % soybean, 10 % non-optimised *Cirina forda* and control diet when compared with other groups. Sodium was significantly higher ($P>0.05$) in group fed 10 % non-optimised *Cirina forda* and lowest in group fed 20 % non-optimised *Cirina forda* while chloride concentration was higher in groups of rats fed 20 % soybean compared to concentration of chloride in rats placed on other supplemented diets.

Table 4.10: Renal function parameters of rats fed with optimized and non-optimised *cirina forda* and soybean supplemented diets

Parameters	Rat Chow (Control diet)	10 % Soybean	20 % Soybean	10 % Optimised *Cirina forda*	10 % Non-optimised *Cirina forda*	20 % Non-optimised *Cirina forda*
UREA	5.050±0.275^{a}	5.750±0.278^{a}	8.925±0.269^{b}	9.225±0.193^{b}	5.475±0.342^{a}	9.525±0.342^{b}
CR	0.750±0.029^{a}	0.875±0.075^{a}	1.875±0.075^{b}	1.975±0.025^{b}	0.775±0.025^{a}	2.375±0.243^{c}
K	3.675±0.138^{b}	4.350±0.466^{b}	2.650±0.155^{a}	2.575±0.149^{a}	3.900±0.168^{b}	2.400±0.041^{a}
Na	107.250±5.648^{ab}	109.750±5.677^{ab}	96.250±1.887^{a}	96.500±1.936^{a}	112.250±7.261^{b}	96.000±1.958^{a}
Cl	100.025±0.931^{ab}	100.825±1.969^{ab}	94.675±1.586^{a}	94.775±1.794^{a}	102.475±2.884^{b}	97.675±2.912^{ab}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

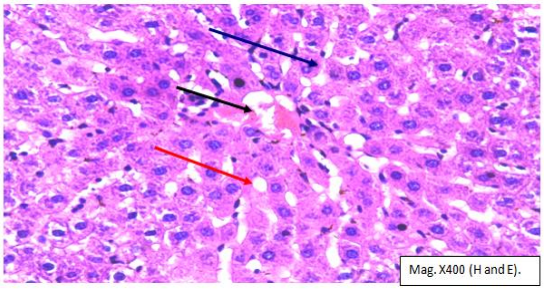
The result in Table 4.11 shows the organ body weight ratios of rats fed with soybean, optimized & non-optimised *Cirina forda* supplemented diets. The Liver/body weight, Lungs/body weight and spleen/body weight of rats fed 20 % soybean protein were not significantly different (P>0.05) from group of animals fed control diet but was higher than that of all the other groups. There was no significant difference (P>0.05) observed in kidney/body weight, heart/body weight and intestine/bodyweight ratios of all the groups.

Table 4.11: Organ/bodyweight ratiosof rats fed with optimized and non-optimised *Cirina forda* and soybean supplemented diets

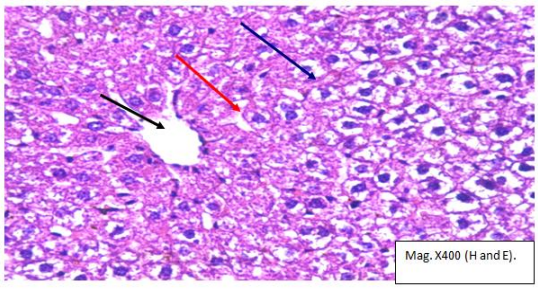
Organs	Rat chow (Control diet)	10 % Soybean	20 % Soybean	10 % Optimised *Cirina forda*	10 % non-optimised *Cirina forda*	20 % non-optimised *Cirina forda*
Liver	5.825±0.275^{b}	5.575±0.180^{b}	5.900±0.334^{b}	4.525±0.272^{a}	4.300±0.091^{a}	5.350±0.065^{b}
Kidney	1.300±0.212^{a}	1.150±0.087^{a}	1.200±0.041^{a}	1.125±0.075^{a}	1.075±0.025^{a}	1.125±0.048^{a}
Heart	0.650±0.029^{a}	0.650±0.087^{a}	0.625±0.025^{a}	0.625±0.025^{a}	0.625±0.025^{a}	0.600±0.000^{a}
Lungs	1.300±0.000^{b}	1.150±0.087^{ab}	1.325±0.103^{b}	1.000±0.000^{a}	1.300±0.000^{b}	1.300±0.000^{b}
Spleen	0.625±0.048^{a}	0.725±0.095^{ab}	1.025±0.125^{c}	0.650±0.029^{a}	0.950±0.087^{bc}	0.700±0.000^{a}
Intestine	4.450±0.946^{a}	5.925±1.161^{a}	5.925±0.440^{a}	5.875±0.633^{a}	4.875±0.676^{a}	4.675±0.232^{a}

Values are mean ± standard errorof triplicate determinations; values on the same row with different superscripts are significant at ($P \leq 0.05$).

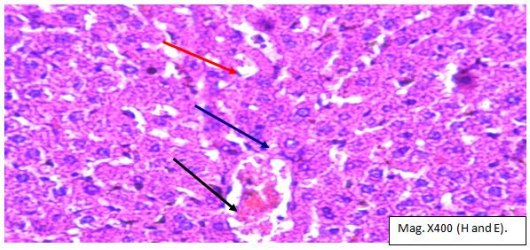
The results in Plates A to F shows the photomicrograph of liver sections of rats fed with different supplemented diets showing central vein (Black arrow) sinusoids (Red arrow) hepatocytes (Blue arrow).



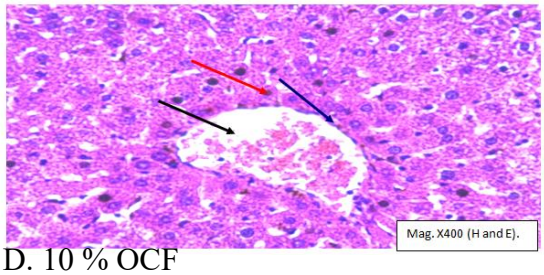
A. Control diet



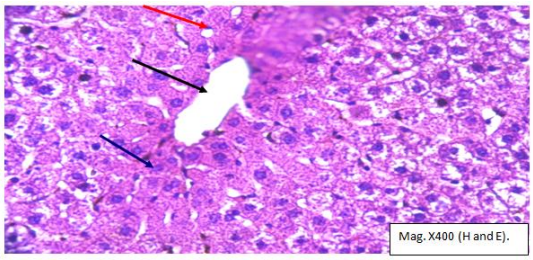
B. 10 % Soybean



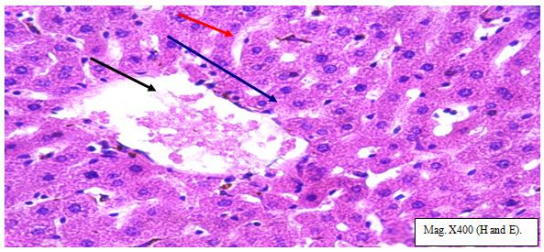
C. 20 % Soybean



D. 10 % OCF



E. 10 % NOCF



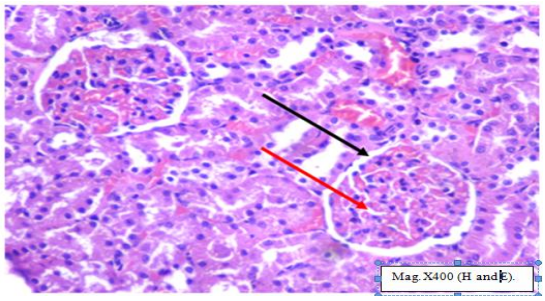
F. 20 % NOCF

Plate I: Photomicrograph of Liver sections of rats fed with different supplemented diets showing central vein (Black arrow) sinusoids (Red arrow) hepatocytes (Blue arrow).

Key

A. Rats fed Rat chow (Control); B. Rats fed 10 % Soybean (SB) protein; C.Rats fed 20 % Soybean (SB) protein;D.Rats fed 10 % Optimised *Cirina forda* (OCF) protein; E. Rats fed 10 % Non-optimised *Cirina forda* (NOCF) protein and F. Rats fed 20 % Non-optimised *Cirina forda* (NOCF) protein.

The result in Plate II (A to F) shows the photomicrograph of kidney sections of rats fed with different supplemented diets showing glomerulus (Red arrow), Bowman's spaces (Black arrow).



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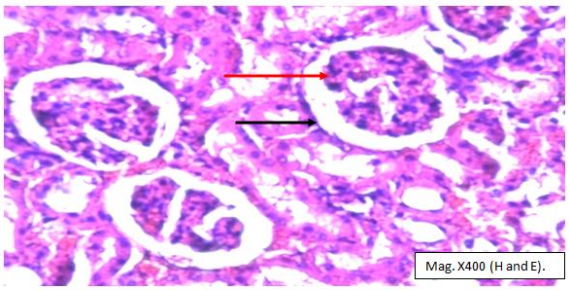
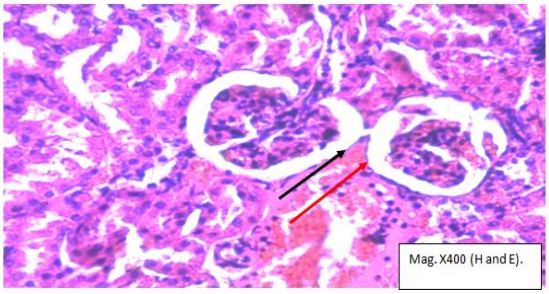
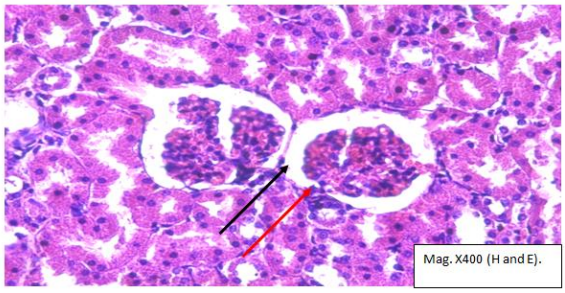


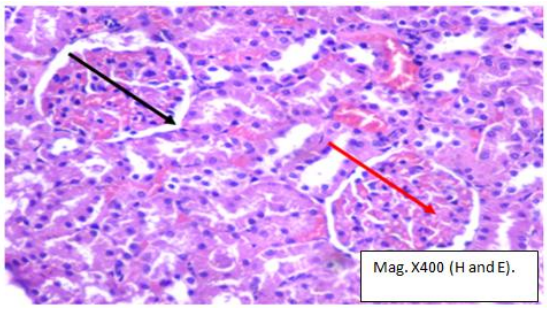
B. 10 % SB



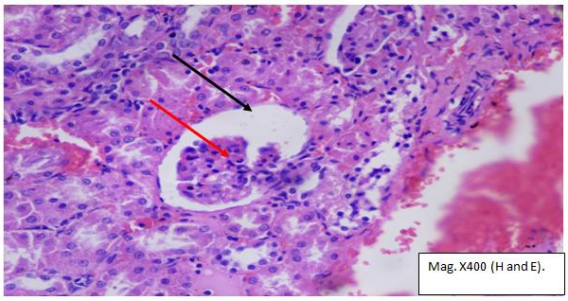
C. 20 % SB



D. 10 % OCF



E. 10 % NOCF

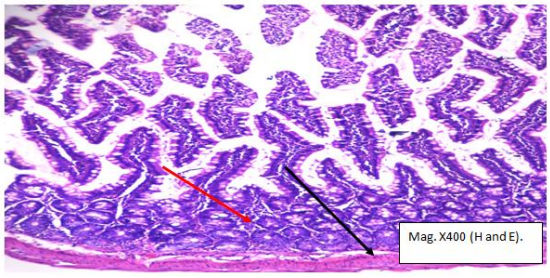


F. 20 % NOCF

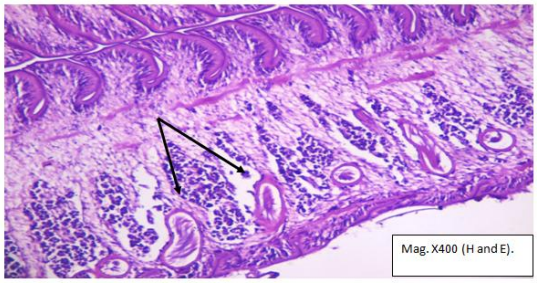
Plate II: Photomicrograph of kidney sections of rats fed with different supplemented diets showing glomerulus (Red arrow), Bowman's spaces (Black arrow).

Key
A. Rats fed normal *control diet* (Control) B. Rats fed 10 % *Soybean* (SB) protein
C.Rats fed 20 % *Soybean* (SB) protein
D.Rats fed 10 % *Optimised Cirina forda* (OCF) protein
E. Rats fed 10 % Non-optimised *Cirina forda* (NOCF) protein

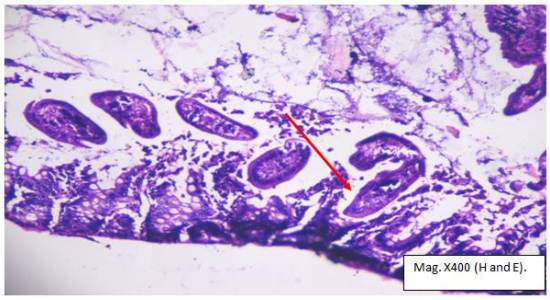
F. Rats fed 20 % Non-optimised *Cirina forda* (NOCF) protein
The results in Plate III (A to F) shows the photomicrograph of duodenumsections of rats fed with different supplemented diets showing crypts (Red arrow), muscularis mucosa (Black arrow).



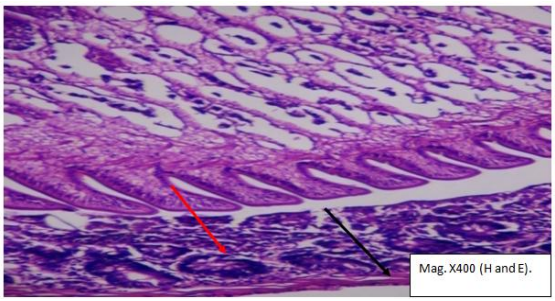
A. *Control diet*



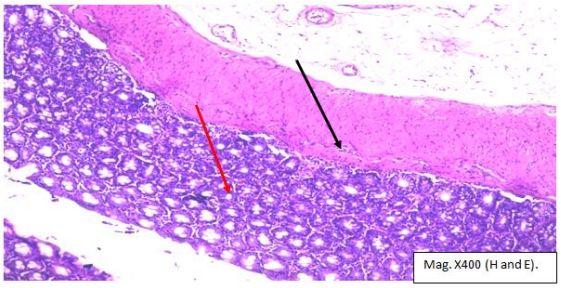
B. 10 % SB



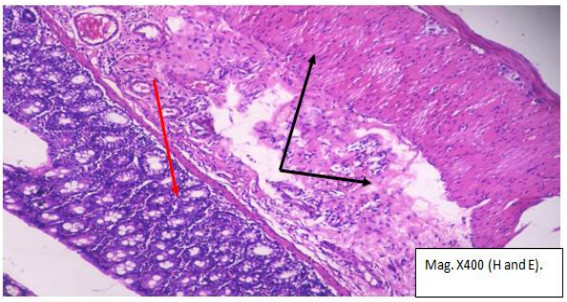
C. 20 % SB



D. 10 % OCF



E. 10 % NOCF



F. 20 % NOCF

Plate III: Photomicrograph of Duodenumsections of rats fed with different supplemented diets showing crypts (Red arrow), muscularis mucosa (Black arrow).

Key
A. Rats fed normal *control diet* (Control) B. Rats fed 10 % *Soybean* (SB) protein
C.Rats fed 20 % *Soybean* (SB) protein
D.Rats fed 10 % *Optimised Cirina forda* (OCF) protein
E. Rats fed 10 % Non-optimised *Cirina forda* (NOCF) protein
F. Rats fed 20 % Non-optimised *Cirina forda* (NOCF) protein

4.2 Discussion

4.2.1 Proximate Composition

The protein value obtained for optimized and non-optimised *Cirina forda* (Table 4.1) is consistent with the report of (Bukkens, 2005) who reported that insects are generally high in crude protein. The significantly low ($P \geq 0.05$) crude protein observed with soybean when compared with optimised *Cirina forda* and non-optimised *Cirina forda* agrees with several studies that the level of protein in insects is generally higher than those of plant seeds (Banjo *et al,* 2006; Defoliart, 2002). The protein content of optimised *Cirina forda* and non optimised *Cirina forda* (51.34 % and 55.35 % respectively) were lower than the values reported by Agbidye *et al*, (2009) (74.35 %) and Oibiokpa, (2017) (64.05 %) for the same insect but higher than that reported by Banjo *et al,* (2006) (20.2 %), Badanaro *et al*, 2014 (51.43 %). The variation in protein content of this insect is presumably mostly due todirences in diets, developmental stage, growth environment, measurement methods or methods of analysis.

The low moisture content obtained for both insect samples is a good attribute for storage. The significantly lower ($P>0.05$) moisture content observed in Soybeancompared to the insects and cassava flakes suggest that soybean may have a longer storage or shelf life. Ogiehor & Ikenebomeh, (2006) reported that lower moisture content corresponds to lower microbial growth and hence longer shelf-life stability and better quality attributes. Values for *Cirina forda* in this study (8.83 ± 0.14 %) are higher than those reported by Oibiokpa (2017) (3.00 %); Banjo *et al*. (2006) (4.40 %); but lower than those reported by Badanaro *et al*. (2014) (10.06 %). The variation shown in moisture content could be attributed to difference in processing methods.

The ash content provides a measure of the total amount of minerals within a food (Nielsen, 2002). Insects are known to be high in calcium, iron and zinc (Van Huis *et al.,* 2013). The percentage ash content of optimised *Cirina forda* and non-optimised *Cirina forda* as well as the soybeans were not significantly different but significantly higher ($P<0.05$) than that obtained for cassava flakes (1.335±0.045), suggesting that the insect under study here can serve as a fair source of mineral for man and animals. The values reported were found to be similar to those reported by Oibiokpa (2017) (7.00± 0.35), but higher than those reported by Banjo *et al.* (2006) (1.50 %), but lower than (10.26 %) reported by Ekop *et al*. (2010)

The values obtained for crude fibre content differed significantly ($P<0.05$) for all the samples with the value in *Cirina forda* being higher than that found in soybean and cassava flakes. This is expected, as insects have been reported to contain significant amounts of fibre. The most common form of fibre in insects is chitin, an insoluble fibre derived from the exoskeleton (Klunder, 2012). The crude fibre content in non optimised *Cirina forda* is significantly ($P<0.05$) higher than that found in theoptimised *Cirina forda*. The variation maybe attributed to the processes involved in the optimisation. The value reported in this study (8.40 %) is lower than that reported by Oibiokpa, (2017) (9.30 %) but higher than those reported by Agbidye, *et al.* (2009) (6.01 %) and Banjo *et al*, (2012) (1.80 %). The physiological role of crude fiber in the body is to maintain an internal distention for properperistaltic movement of the intestinal tract (Oduor *et al*., 2008). A diet very low in fiber, could therefore lead to constipation which might bring discomfort to the body system (Groff *et al*., 1999). Diets with high fiber content have been used for weight control and fatreduction, as they give a sense of satiety even when small food is eaten (Ekop, 2004).

Edible insects are a good source of fat.The fat content obtained for *Cirina forda* in this study (9.01 %)was low when compared withthat reported by Agbidye *et al.* (2009) (14.3 %), Oibiokpa (2017) (12.50 %), Banjo *et al*. (2006) (14.20 %), and Cerritos (2009) (22 %) for the same species of insect. The fat content of soybean was significantly higher ($P<0.05$) than all other samples with cassava flakes being significantly lower ($P> 0.05$). The higher crude fat observed in optimised *Cirina forda* insect is most likely as a result of the optimization process.

According to Van Huis *et al.* (2013); Bukkens (1997); Bukkens (2005), insect oils are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids. The nutritional importance of these two essential fatty acids is well recognized, mainly for the healthy development of children and infants (Michaelsen *et al.,* 2009). Some insects can also provide a higher caloric contribution to the diet than soy, maize, or beef (Gahukar, 2011). These lipids provide a ready source of energy for the body and are also essential in diets as they increase palatability of food.

The low carbohydrate content found in *Cirina forda* (Table 4.1) is also in agreement with reports that insects are generally low in carbohydrate (Ekop *et al*., 2010; Oibiokpa, 2017). The significantly ($P<0.05$) higher carbohydrate content observed in cassava flakes is expected and in agreement with previous reports that it is the third source of food carbohydrate after rice and maize (Yang *et al.,* 2012). Cassava flakes has played a vital role in the diet of many African countries as a major source of low cost carbohydrate (Jansson *et al*., 1990; Enidiok *et al*, 2008). According to Bednářová (2013), high protein insect species have lower energy content. This is evident in this study, thus even though *Cirina forda* may

not be low in carbohydrate, it is not eaten as a main diet since human adult need about 400 – 500 g carbohydrate intake as starch (Ekop *et al*., 2010).

4.2.2 Anti-nutritional composition

Anti-nutrients such as hydro-cyanide, oxalates, phytates and tannins have been reported in edible insects in Nigeria (Ekop *et al.,* 2010; Ifie and Emeruwa, 2011) and also in most legumes and grains.

Cyanogenic glycosides were found to be below the maximum permissible limit for all the samples (WHO, 2003). The observed levels of the cyanogenic glycosides were in conformity with those reported by Ekop *et al*. (2010) for *G. lucens* (2.187 mg/100g)*, H. meles (*2.734/100g)*, R. ph*oenicis *(*2.422/100g) and *Z. variegates* (3.203/100 g) respectively. Cyanogenic glycosides are highly toxic to animals and high levels have been implicated in cerebral damage and lethargy in man and animals (Akyildiz & Vuran, 2010). The cyanogenic glycoside content of samples in this report(3.513 mg/100g, 2.363 mg/100g, 3.923 mg/100g and 0.04 mg/100g respectively) are below the lethal dose/toxicity level reported by Ekop (2010) (35 mg/100g).

The phytate content of insect samples in this report were below the permissible levels of 22.10 mg/100g (WHO, 2003) except for the cassava flakes (29.047 mg/100g) and soybean (210.707 mg/100g) which were much higher. This could be attributed to the specie of these samples used or the processing method as reports by Ekop (2004) has shown that toxic component like phytic acid are easily detoxified during processing like – frying, boiling and roasting. The observed level in *Cirina forda* (16.747mg/100g) inthis study is much lower than that reported by Ifie and Emeruwa (2011) for *Oryctesmonoceros* (178 mg/100g), but higher than the range of 0.28 to 0.289 mg/kg by Ekop *et al*. (2010) for four edible insects

(*G. lucens* (cricket)*, H. meles* (yam beetle), *R. phoenicis* (palm weevil) and *Z. variegatus* (grasshopper). Phytic acid has been implicated in the removal of phosphorus and causing indigestion and flatulence in human system (Ndubuakaku *et al*., 1998). Considering the low values of phytic acid *Cirina forda*, means thatthis insect could be consumed without any fear of phytic acid toxicity to the human body.

Saponin that have been shown to lower plasma glucose, insulin and (or) plasma cholesterol and triacylglycerols (Slavin *et al*., 1999). Saponin was the most abundant antinutrient observed in the insect and plant samples under study. The amounts were above the permissible limit 48.05 mg/100 g (WHO 2003; Aletor, 1995) in all the samples except in cassava flakes (32.48 mg/100 g). This could be implicated to the feed consumed by this insect and also the method of processing adopted for soybean and insect. Ndukwe *et al*. (2007) and other researchers have reported the Phytochemical screening of the plant (*Vitellaria paradoxa*) parts (stem, leaves, bark) revealed the presence of saponins, tannins, alkaloids, terpenoids and carbohydrates (free reducing sugars, ketoses, pentoses and starch) of which *Cirina forda* is a known defoliator of its leaves.

Oxalates are known to sequester and precipitate some useful metallic elements, thus making them unavailable for adsorption in human system (Groff *et al.,* 1995). The observed values for oxalate (2.520 mg/100g; 2.790 mg/100g; 13.750 mg/100g and 2.253 mg/100g) in this study for non-optimised *Cirina forda,* optimized *Cirna forda,* cassava flakes and soybean respectively were far below the lethal dose reported to be between 200 mg/100g to 500 mg/100g (Pearson, 1973). Hence, with processing, *Cirina forda* is safe for consumption with respect to oxalate toxicity.

Tannins possess both toxic and therapeutic functions. The presence of tannins reduces the protein digestibility of food by inhibiting the activity of trypsin and chymotrypsin. They are toxic in that they coagulate protein (Groff *et al*., 1995). They have been reported to be responsible for decreases in feedintake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimentalanimals (Chung *et al*, 1998). Tannin toxicity can be removed by heating. Okon and Ekop (2008) had shown that boiling and fermenting can drastically reduce tannin in cowpea. Since the amount of tannins in all the samples were below the permissible limits (76-90 g/Kg) (WHO, 2003; Aletor, 1995), the presence of this antinutrient may not affect the digestibility of proteins obtained from these insects and plant samples.

4.2.3 Mineral composition

Minerals play an important role in biological processes. Micronutrient deficiencies, which are commonplace in many developing countries, can have major adverse health consequences, contributing to impairments in growth, immune function, mental and physical development (Turan *et al.,* 2008). The high potassium level in all the samples (Table 4.3) is of significance because potassium is a cofactor in energy metabolism, glycogenesis and cell growth. Potassium has also been shown to play a role in the treatment of coronary heart disease as increased intake reduces blood pressure by increasing the excretion of sodium (Gibney *et al*., 2009).

Phosphorus was observed to be the most abundant mineral in both optimised *Cirina forda* and non-optimised *Cirina forda* and second after potassium in soybean and cassava flakes. Phosphorous functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids (Adedapo *et al.,* 2007). It is

involved in the synthesis of phospholipids and phosphoproteins. Practically, every form of energy exchange inside living cells involve the forming or breaking of high-energy bonds that link oxides of phosphorus to carbon or to carbon-nitrogen compounds (Hays & Swenson, 1985).

Calcium is essential for absorption of vitamin B12 from the gastro-intestinal tract and also associated with phosphorus for growth and maintenance of bones, teeth and muscles (Turan *et al*, 2003). Calcium was significantly higher ($P<0.05$) in the soybean sample compared to other samples. The significantly higher calcium in non-optimised *Cirina forda* compared to the optimized form of *Cirina forda* may be due to differences in habitat, feeding, developmental stage of harvest. Deficiency of phosphorus-calcium balance results in osteoporosis, arthritis, pyorrhea, rickets and tooth decay. Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles (Turan *et al.,* 2003).

Magnesium is associated with activation of various key enzyme systems, like kinases and is an essential component of bone, cartilage and the crustacean exoskeleton (Kayode *et al*, 2013). The highest magnesium concentration (Table 4.3) was observed in soybean (264.33 mg/100g) which is less than, the average requirement for magnesium intake (300–400 mg/ day) in humans depending on gender, age, body habitus, physiologic need as well as individual variation in absorption (intestinal and renal) and excretion (Abdulsahib, 2011). The high level of this mineral in soybean is expected and in agreement with reports of Yang *et al.* (2014) which state that dietary intake of magnesium-containing foods such as peas, beans, spinach, nuts, seafood, vegetables, seeds is generally sufficient or may be supplemented by other sources, that is, vitamins and/or minerals.

Iron essential in transportation of oxygen and electron within the body). Iron content of the mopane caterpillar vary between 31 and 77 mg per 100 g of dry weight, calcium content of G. sigillatus, S. gregaria, and T. molitor contain (130, 70, and 40 mg/100 g respectively). zinc content S. gregaria contains 18.6 mg/100 g, G. sigillatus – 13.9 mg/100 g, and T. molitor – 11.2 mg/100 g. The palm weevil larvae (Rhynchophorus phoenicis), contain 26.5 mg/100 g of Zinc (Bukkens, 2005). This is in agreement with the reports of Van Huis, (2003) who reported that the nutritional value of edible insects is highly variable. Even within the same group of species, the nutritional value may differ depending on the metamorphic stage of the insect, its living habitat, and its diet (Van Huis, 2003).

4.2.4 Amino acid composition

The insect samples and soybean sample were found to contain 18 naturally occurring amino acids and may therefore be good sources of both essential and non essential amino acids. Protein quality, and hence the nutritiolnal value of food is determined by its amino acid composition and the digestibility of the food. Theessential amino acids (EAA) cannot be synthesized in the body and must be taken in the diet (Guoyao *et al.,* 2014). The results presented in Table 4.5 shows that the optimized *Cirina forda* contained a higher concentration of all the essential amino acids (in contrast to the concentration obtained for the non-optimised *Cirina forda* but lower than the EAA concentration of most ofthe EAA in soybean except for leucine, methionine, Arginine and threonine which were lower in concentration. The variations in the concentration of EAA in *Cirina forda* may be due to developmental stages of the insect, diet, habitat or the effect of optimization.Among the EAA obtained in this study, leucine was present in the highest amount in both soybean and *Cirina forda* (optimized &non optimized). Nevertheless, when compared with the

WHO/FAO/UNU pattern (WHO, 2007) both Optimised and non-optimised *Cirina forda* amino acid profile covered and exceeded the requirement except for the content of lysine in non-optimised *Cirina forda* form which was found to be slightly lower than the requirement (45 mg/g).

Leucine plays an important role in metabolic regulations and intracellular signaling. Exercise produces changes in protein and amino acid metabolism. These changes include degradation of the branched-chain amino acids, production of alanine and glutamine, and changes in protein turnover. Leucine is one of the branched chain amino acid most affected by exercise (Layman, 2002). Leucine is known to interact with the insulin signaling pathway to stimulate downstream signal control of protein synthesis, resulting in maintenance of muscle protein during periods of restricted energy intake. Leucine also appears to modulate insulin signaling and glucose use by skeletal muscle. Whereas total protein is important in providing substrates for gluconeogenesis, leucine appears to regulate oxidative use of glucose by skeletal muscle through stimulation of glucose recycling via the glucosealanine cycle. These mechanisms produce protein sparing andprovide a stable glucose environment with low insulin responses during energy-restricted (Layman & Walker, 2006).

Lysine is one of the two strictly indispensible amino acids whose main role is to participate in protein synthesis. In mammals, catabolism of lysine through the saccharopine pathway also results in the irreversible formation of glutamate and a-aminoadipate, which are then subjected to deamination and oxidation. In addition to its importance as a precursor for protein synthesis, lysine is also a precursor for the biosynthesis of carnitine, which plays an important role in b-oxidation (Van *et al.,* 2000).

Valine on the other hand is one of the branched chain amino acids involved in diverse physiological and metabolic functions. It is required for proper immune functions, energy production, and synthesis of proteins and neurotransmitters (Monirujjaman & Ferdouse, 2014).

Thus, the Optimised *Cirina forda* and non-Optimised *Cirina forda* forms of *Cirina forda* and soybean represent a valuable alternative protein source and provide satisfactory amounts of the required essential amino acids.

Glutamic acid and aspartic acid were the highest concentrations of Non Essential Amino Acids (NEAA) as shown in Table 4.6 this is similar to the reports of Solomon & Prisca, (2012), Ekpo & Onigbinde (2005), Oibiokpa (2017) for *Lepidoptera litoralia* (12.50 and 10.6 mg/100g) and *R. phoenicis* larva (12.91 mg/100g and 7.02 mg/100g) respectively. Oibiokpa (2017) reported lower values (8.94 mg/100g and 7.82 mg/100g) for same species. Glutamate and Aspartate are both known to regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of the organisms (Wu 2010 & 2013). They also hold great promise in prevention and treatment of metabolic diseases (example, obesity, diabetes, and cardiovascular disorders), intra uterine growth restriction, infertility, intestinal and neurological dysfunction, and infectious disease (Wu, 2013). Glutamic acid plays an important role in amino acid metabolism because of its role in transamination reactions and is necessary for the synthesis of key molecules, such as glutathione which are required for removal of highly toxic peroxides and the polyglutamate folate cofactors while Aspartic acid is the precursor of AAs methionine, threonine, isoleucine, and lysine and regulates the secretion of important hormones (Wang et al, 2013). The least predominant NEAA was found to be Cysteine. Observed result is lower (1.45

mg/100g) than that reported by Zielinska *et al*. (2018) (3.60 mg/100g); Ekpo & Onigbinde (2015) (2.20 mg/100g) but higher than that reported by Oibiokpa (2017) (0.66 mg/100g).

Soybean contained higher amounts of total sulphur containing amino acids (TSAA) (methionine and cysteine) as compared to the optimized and non-optimised C*irina forda* as shown in Table 4.7. The Sulphur containing amino acids are very important sources of sulphur. Methionine in the form S-adenosyl methionine is required for transmethylation reactions (Rubin *et al.,* 2007). Methionine is essential for treating liver disorders, improving wound healing, and treating depression, alcoholism, allergies, asthma, copper poisoning, radiation side effects, schizophrenia, drug withdrawal, and Parkinson's disease (Mischoulon & Fava, 2002). It is also important for antioxidants and protein synthesis as well as the synthesis of lipotropic compounds like taurine, gluthathione, choline, carnitine and s-adenosyl methionine (Kiruthikajothi *et al*., 2014). On the other hand, Cystein is is an important detoxicant of specific substances and an important component of the antioxidant in cells, gluthathione. The TSAA values obtained in this work for non-optimised *Cirina forda* (2.87 mg/100g)is higher than that reported by Oibiokpa *et al*. (2018) for the same insect (0.51 mg/100g), Cricket (*Gryllus assimilis*) 1.37 51 mg/100g and Grasshopper *(Melanoplus foedus*) 1.11 mg/100g.

The aromatic amino acids (TArAA) phenylalanine and tyrosine were found to be higher in Soybean compared to non-Optimised and optimised *Cirina forda*. Tyrosine is required for the synthesis of certain hormones such as thyroid hormone, epinephrine and norepinephrine and a pigment, melanin (Fernstrom & Fernstrom, 2007). The selected insects contained sufficient amount of phenylalanine and tyrosine to meet the requirement for preschool

children (2-5yrs), school children (10-12yrs) and adults (18+yrs) being (63 mg/g protein, 22 mg/g protein and 19mg/g protein respectively) (FAO/WHO/UNU, 1985).

Figures 4.1 & 4.2 clearly indicate that there is a direct correlation between feed intake and weight gain.Highweight gain has been attributed to high feed intake in experimental animals. The high feed intake of rats fed with 20 % soybean supplemented diets correlates with their apparent weight gain. This could be attributed to soybean palatability and excellent amino acid compositionwhen compared to the control diet and diets supplemented with *Cirina forda*. The lowfeed intake of rats fed with 20 % non-Optimised *Cirina forda* supplemented diet is in agreement with the findings of Ande (2003), who reported that rats fed *Cirina forda* diet had lower feed intake as compared to rats fed skimmed milk diet (control diet). Low feed intake has been attributed to deficiency or imbalance of amino acids, presence of antinutrients in the protein source or unpalatability of the diets (Ekpo, 2011). Jacquot and Peret (1972) reported that the nature of proteins included in diets influences the appetite of animals, and may cause significant reductions in food intakes during *ad libitum* feeding. In addition, Passmore *et al.* (1974) reported that if the total energy intake is inadequate, some dietary protein is used for energy, and is therefore not available to satisfy protein needs for growth and weight gain.

4.2.5 Haematological parameters

Haematological parameters are good indicators of the physiological, nutritional and pathological status of animals. They are also valuable in monitoring feed toxicity (NseAbasi *et al.,* 2014). It can also be used to determine the extent of deleterious effect of foreign compounds on the blood of the albino rats (Odeyemi *et al.,* 2009) as well as to explain blood relating functions of a given feed formulation (Ajayi *et al.,* 2005).

There was a general increase in the Hb, PCV and RBC of rats fed 20 % soybean and 20 % non-optimised C*irina forda* when compared to the control group (Table 4.6). A high level of Hb, PCV and RBC is an indication that the rats are not anaemic while a lower level is a sign of anaemia (Aboderin*, et al.*, 2006). Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al.*, 2012; Soetan *et al.,* 2013; Isaac *et al.,* 2013). However, Olaleye *et al*. (1999) reported a decrease in hematocrit and hemoglobin concentrations of rats fed differently processed Soybean diets. The reason for the disparities in haemoglobin in two studies could be attributed to the methods of preparation of the Soybean diets. While the present study has used only raw soybean which was dried at room temperature, the report of Olaleye *et al*. (1999) used sun-dried raw soybean, boiled soya bean, roasted soya bean and alkali treated soya bean diets.

Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) indicates blood level conditions (Aster, 2004) and usually aid the characterization of anemic states in animals (Aiello *et al*, 1998). The significantly higher ($P<0.05$) MCV and MCH in all the groups of rats with exception of groups fed 10% non-optimised *Cirina forda* when compared with the control group is an indication that the animals were not anaemic (Ukonu *et al.*, 2018).

Total White Blood Cell (WBC) are capable of fighting infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response (NseAbasi *et al*., 2014). A significantly lower ($P>0.05$) TWBC was observed in the group of rats fed 10 % optimized C*irina forda* protein.

Animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan *et al*., 2013). However the TWBC is considered to be within normal range of (5 – 13 (x109/l) according to Burke (1994).

The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Malomo, 2000), assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu *et al.,* 2003). Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Yakubu *et al*., 2006).

Aminotransferases, which include alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are involved in the transfer of an amino group from a 2-amino acid to a 2-oxoacid with the aid of pyridoxal phosphate as a cofactor for optimal activity. A rise in any of these is an indicator of damage to cystolic and/or mitochondrial membranes (Crook, 2006).

AST is present in high concentrations in hepatic, renal, cardiac, and skeletal muscle cells and erythrocytes; hence damage to any of these tissues may increase plasma AST levels (Saka *et al.*, 2011; Ige *et al.*, 2011). The significantly higher ($P < 0.05$) AST and ALT activity observed in the serum of rats in all the study groups with exception of AST in group fed 10 % soybean may not necessarily be as a result of liver damage. Oibiokpa *et al.* (2018) and Ekpo (2011) who fed rats with insects supplemented diets low in protein and reported elevated levels of AST and ALT. Therefore, increased serum activity of ALT may

be ascribed to low protein quality of the various supplemented diets, poor feeding (unpalatiability) or length of feeding (Ekpo *et al.*, 2011).

Alkaline phosphatase (ALP) is present in high concentrations in the bone, mucosa of the small intestine, kidney, liver and plasma. Damage in any of these organs may cause a release of ALP into the bloodstream. ALP is also a marker for the plasma membrane and endoplasmic reticulum used to assess the integrity of plasma membrane and endoplasmic reticulum (Akanya *et al.,* 2014). An increase in serum ALP activity is also associated with bone growth (Ramaiah, 2007). The significant increase in serum ALP activity observed in the group of rats fed, 10 % optimized, 10 % non-optimised and 20 % non-optimised *Cirina forda* diet may not be as a result of liver damage, but might be associated with bone growth since the rats were young and actively growing (Adeyemi *et al.,* 2015).

The concentration of total protein and albumin in serum are indicators of the synthetic ability of the liver (Singh *et al*., 2011). Decreased concentrations of serum albumin and total protein are therefore markers of impaired liver function due to decreased ability of the liver to synthesize proteins. Concentration of albumin in serum is also used to monitor the protein / nutritional status of animals since the synthetic rate of albumin is dependent on protein intake (Burtis *et al*., 2008).

In this study, total protein concentration were significantly lower ($P<0.05$) in group of rats fed 20% non-optimised *Cirina forda* and significantly higher in groups fed 10 % and 20 % soybean protein when compared with the control group.The higher concentration of total protein observed in rats fed soybean diet may also be due to a higher bioavailability of essential amino acids required for synthesis of proteins. Similar results was reported by Oibiokpa *et al*. (2018) for *Cirina forda* (6.17 ± 0.88 g/dL) and (6.43 ± 0.09 g/dL) and

Akinnawo *et al*. (2002) after administration of raw extracts of the same insects in rats as (6.4 g/dL for 1,750 mg/kg concentration of extracts; 6.7 g/dL for 2,250 mg/kg and 7.0 g/dL for 3,750 mg/kg).

Serum albumin concentration was found to be lower in group of rats fed with 10% and 20% soybean as well as 20 % non–optimised *Cirina forda* supplemented diets when compared to rats fed with contol diet. No significant difference ($P>0.05$) was observed in the Total protein and albumin of groups placed on 10 % optimised and 10 % non-optimized *Cirina forda* protein when compared with the control group.

Serum total and conjugated bilirubin (B1 & B2) were significantly higher ($P<0.05$) in all the test groups when compared with the control group. Plasma levels of bilirubin have been extensively measured in various clinical conditions. While very high levels of bilirubin are certainly neurotoxic, numerous investigations indicate that mildly elevated levels of bilirubin can be beneficial (Rigato et al 2005; Sedlak and Synder, 2004). For instance, elevated serum levels of bilirubin have been reported to be associated with diminished risk of coronary artery disease (Djousse *et al.,* 2003).

Measurement of blood and plasma concentration of urea, creatinine and electrolytes are useful indicators of the kidney function (Ukonu *et al.*, 2018) although several factors such as excessive protein intake, shock, gastrointestinal hemorrhage etc. could also be a contributing factor. Urea is a waste product of protein and amino acid catabolism which is filtered through the glomerulus and excreted majorly in the urine. An increase in plasma concentration of urea occurs majorly as a result of impaired renal function (Ashafa *et al*., 2009).

The urea concentration of the study groups fed 20 % soybean, 10 % optimized *Cirina forda* and 20 % non optimized *Cirina forda* when compared with the control group was found to be much higher but still within the normal range of blood urea nitrogen (BUN) in males (19.1-31.7 mg/ dl) and in females (18.1-28.9 mg/ dl) (Kamal, 2014). This may imply that there was no impairment to glomerular filtration. This observation is supported by the report of Ekpo (2011) that blood urea level of rats fed with insect/larval diets was also comparable to rats placed on control diets.

Creatinine is a breakdown product of phosphocreatine, a high energy compound utilised during muscle contraction. Creatinine is freely filtered by the glomerulus; therefore a build up in plasma levels of creatinine is an indicator of kidney function impairment (Burtis *et al*., 2008). The significant difference ($P<0.05$) in serum concentration of creatinine in rats fed 20 % soybean, 10 % optimised *Cirina forda* and 20 % non-optimised *Cirina forda* were not above the normal range (Kamal, 2014) implies that the supplemented diet may not have detrimental effect on the kidney. And differences may be as a result of other factors such as excessive protein intake (Kamal, 2014).

A significant increase in serum electrolytes is usually an indication of tubular and glomerular dysfunction (Ashafa *et al*., 2009) but the serum concentrations of electrolytes (Sodium, potassium and chloride) in rats fed with the two forms of *Cirina forda* supplemented diets were also similar to rats fed with the standard control diets, therefore supplemented diet did no lead to kidney dysfunction.

Organ weight is one of the most sensitive indicators of an effect of test diet, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey *et al.,* 2004).

The organ /body weight ratios of the kidney, heart and intestine of animals placed on supplemented diets were similarwith those of rats fed the control diet. This suggests that the various supplemented diets did not cause any adverse damage to these organs. Howeveer there were variations observed in the organ/body weight of the liver, lungs and spleen in some groups fed different supplemented diets.

Histological analysis is the gold standard for tissue examination, either for research or diagnostic purposes, for both qualitative and quantitative measure. It is used to assess the inflammation or healing stage and to monitor the presence and distribution of degradation products that dissolved into the surrounding tissue (Ragamouni *et al*., 2013). In the current study, histopathological examination of examined organs (liver, kidney, and intestine) revealed no distinct change in the histoarchitecture when compared with the control. This is similar to the results of Akinnawo *et al*. (2002) who reported that extracts of processed *Cirina forda* did not alter the histoarchitecture of the liver and hearts of rats. In this study, the group fed 20 % non optimized *Cirina forda* showed signs of mild distortion of liver central vein, cellular morphology, renal capuscle and muscularis mucosa of the duodenum. This may be attributed to the high level of insect protein inclusion in the diet of this group. The findings were very similar to the findings of Caimi *et al*. (2020) who observed that the highest inclusion level of Hermetia illucens larva meal (H) led to changes in oxidative stress biomarkers.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

It can be conclude from this study that the optimized and non-optimized forms of *Cirina forda* were found to be good sources of protein and fat but were generally low in minerals. It was also observed that optimization of *Cirina foda* did not significantly alter the nutrient composition of the insect.The antinutrients contained in the insects were found to be below permissible limits.The two forms of *Cirina forda* were also found to be rich in essential amino acids, non essential amino acids, saturated fatty acids and unsaturated fatty acids. The lesser weight gain in rats placed on *Cirina*forda diets compared to that of the soybean may be due to low feed intake and difference in acceptability of the diets. *Cirina forda* supplemented diets did not alter the biochemical parameters and the histo-architecture of organs. Inclusionof optimized and non-optimized *Cirina forda* in the diets of rats did not have any adverse effect on serum AST, ALT, ALP, Urea, creatinine, electrolytes, albumin, bilirubin and total protein, haematological indices and histoarchitecture of the various organs. *Cirina forda* may therefore be relatively safe for consumption and may be a promising alternative to conventional animal proteins due to its high essential amino acid. Therefore, the inclusion of insects in human diets can reduce the burden and over dependence on other conventional animal proteins if the problem of acceptability is completely addressed.

5.2 Recommendations

This research work has been able to establish that *Cirina forda* is a promising source of protein and other macro nutrients and is safe for consumption and therefore could help in mitigating protein energy malnutrition. Further research however should be carried out in the following areas.

1. The optimization parameters should be further developed as to provide a better enabling environment to reduce mortality of the insect. This will enable all year round availability of the insect.
2. The biological evaluation of the insect formulated diets (true digestibility (TD), Net Protein Utilization (NPU), Biological Value (BV), Protein Energy Ratio (PER), Protein Amino Acid Score (PAAS) shoud also be carried out as this will give us the necessary information on the functional quality of the diets.

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APPENDIX A

WEEKS	Rat Chow	10 % Soyabean protein	20 % Soyabean protein	10 % Optimised *C. forda* Protein	10 % Unoptimised *C. forda* Protein	20 % Unoptimised *C. forda* Protein
Week 1	$51.931\pm0.877^{c}_{b}$	$40.161\pm0.696^{a}_{a}$	$49.983\pm0.554^{c}_{a}$	$44.144\pm0.745^{b}_{a}$	$45.181\pm0.763^{b}_{a}$	$40.737\pm0.661^{a}_{a}$
Week 2	$49.336\pm0.833^{c}_{a}$	$40.564\pm0.703^{a}_{a}$	$54.983\pm0.609^{d}_{b}$	$45.909\pm0.775^{b}_{b}$	$47.440\pm0.801b^{c}_{b}$	$40.941\pm0.664^{a}_{ab}$
Week 3	$54.531\pm0.921^{c}_{c}$	$41.369\pm0.717^{a}_{b}$	$57.481\pm0.637^{d}_{c}$	$47.673\pm0.804^{b}_{c}$	$49.247\pm0.831^{b}_{c}$	$41.960\pm0.681^{a}_{b}$

Values are means of daily feed intake of the animals and were reported as mean ± standard error of means (SEM). Values were compared using 2-ways ANOVA. Values along the row with different alphabetic superscripts are significantly difference at $p\leq 0.05$. Also, values along the coloumn with different alphabetic subscripts are significantly difference at $p\leq 0.05$.

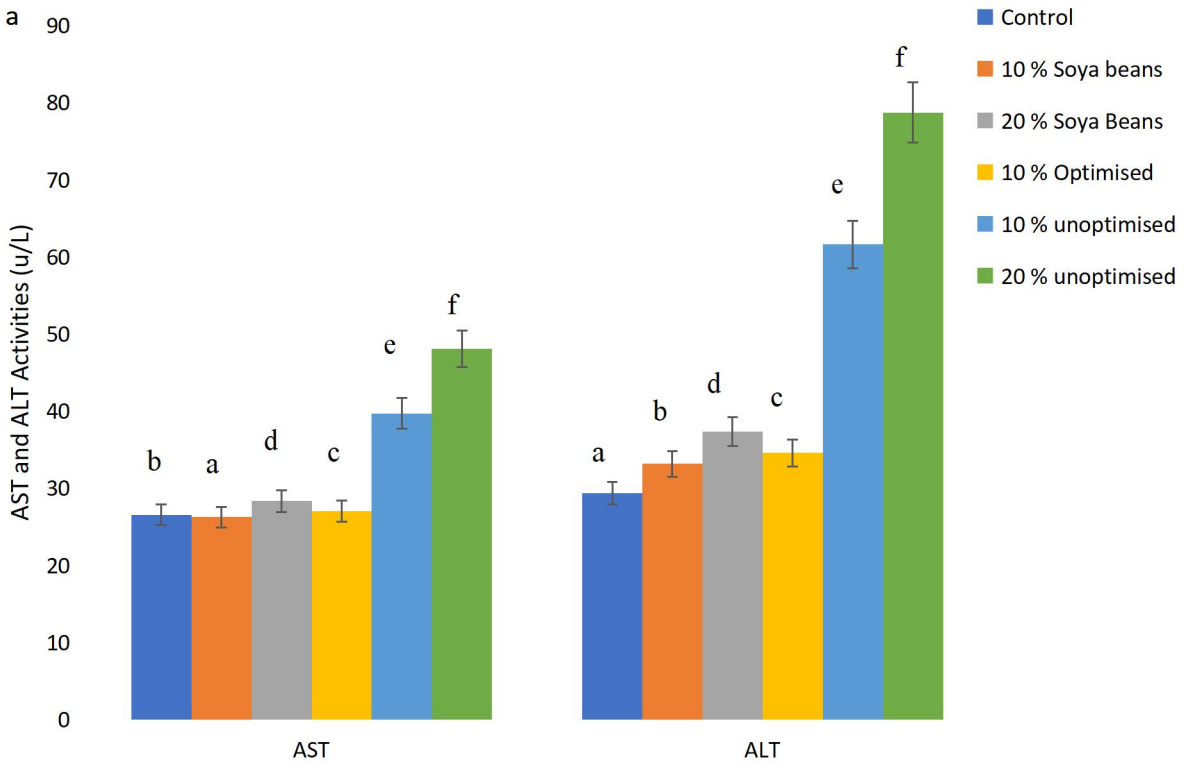
Appendix A: Weekly Feed Intake of the Experimental Animal for the 3 Week Period

APPENDIX B

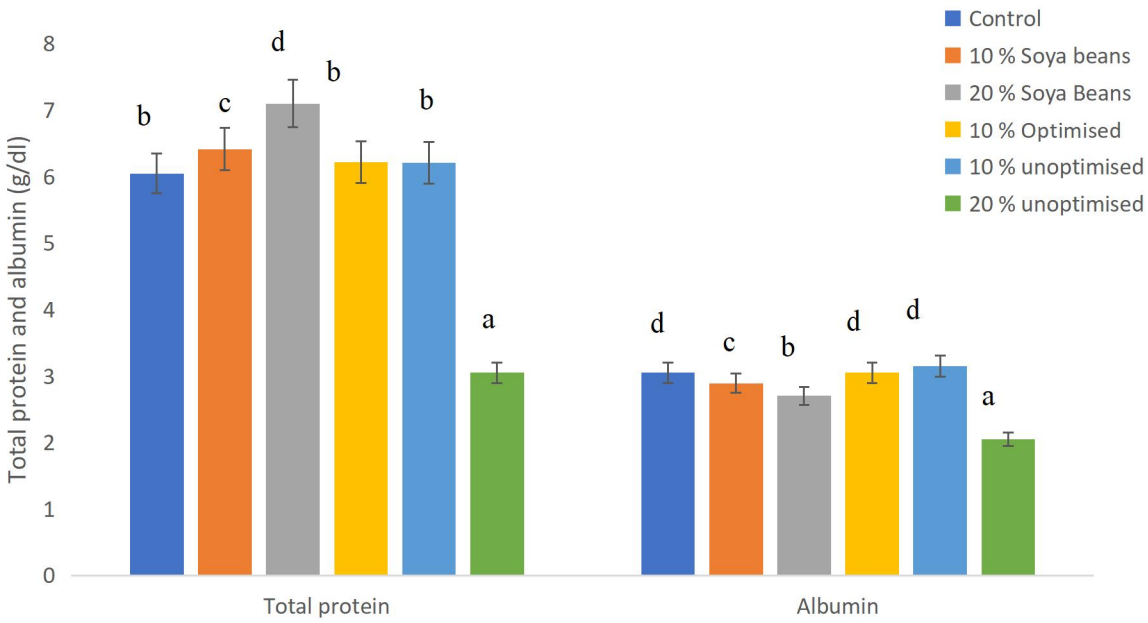
Groups	Rat Chow	Soyabean protein 10 %	Soyabean protein 20 %	10 % Optimised *C. forda* Protein	10 % Non-optimised *C. forda* Protein	20 % Non-optimised *C. forda* Protein
0	0	0	0	0	0	0
1	2.393	0.97	4.043	1.9	2.843	-5.66
2	4.337	1.46	13.99	3.55	3.423	4.3
3	7.973	4.987	26.12	4.743	4.833	5.433

Appendix B: Weekly Weight gain

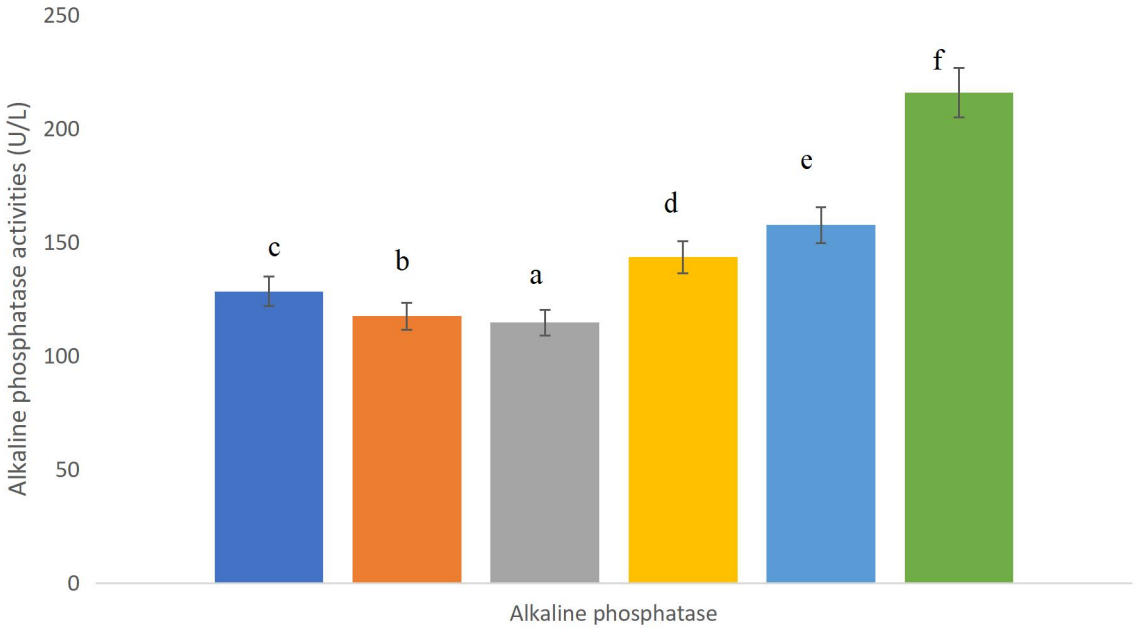
APPENDIX C



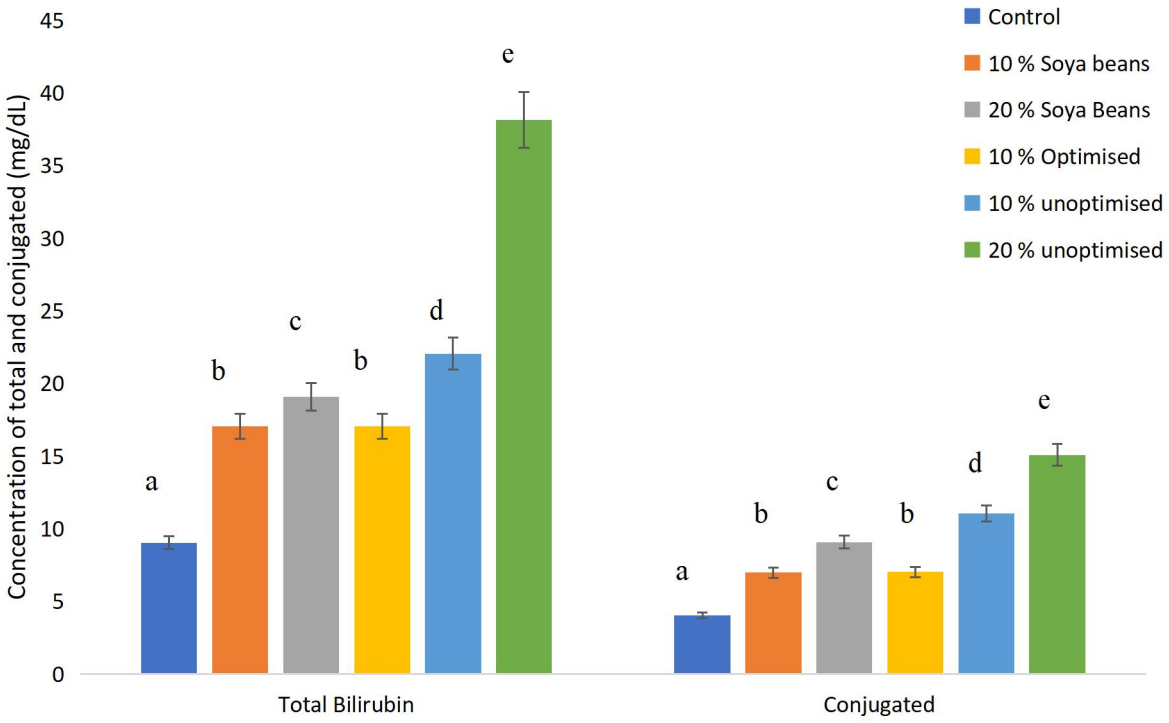
Appendix C1: Serum enzyme activities (AST and ALT) of rats fed different supplemented diet



Appendix C2: Total protein and Albumin of rats fed different supplemented diet

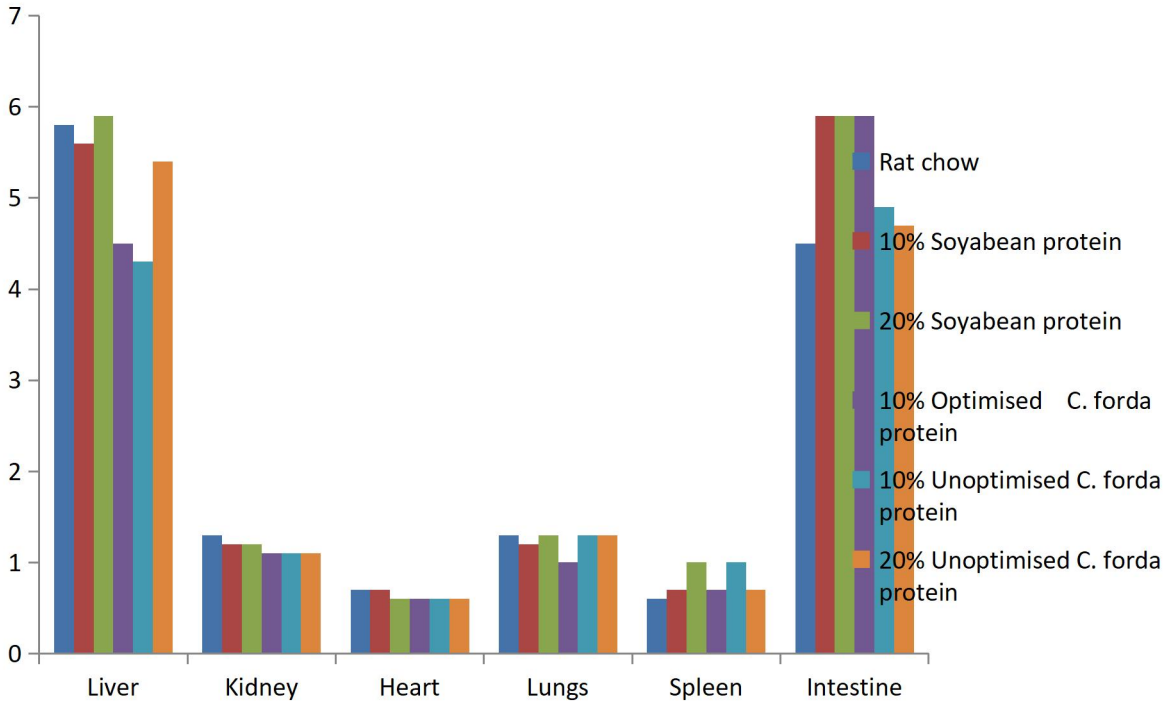


Appendix C3: Serum enzyme activity (ALP) of rats fed different supplemented diet



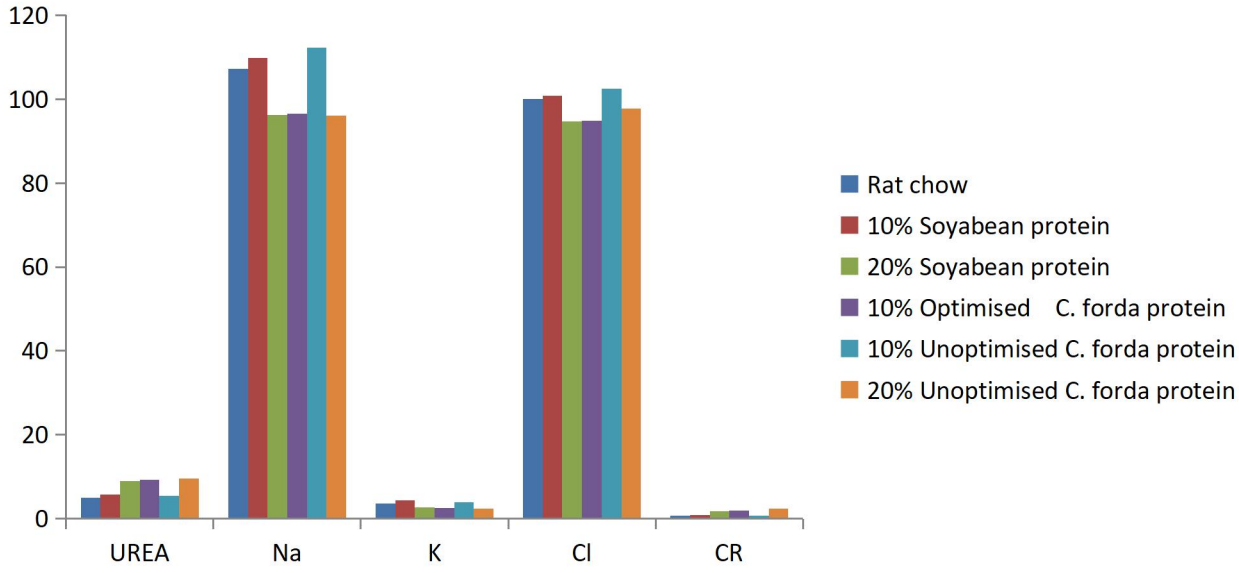
Appendix C4: Total Bilirubin and Conjugated protein of rats fed different supplemented diet

APPENDIX D



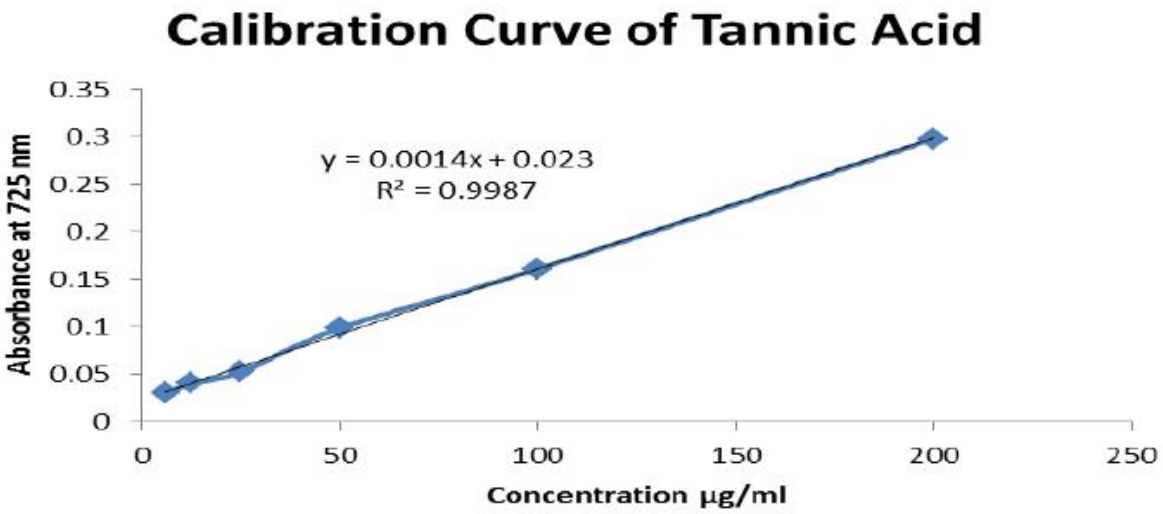
Appendix D: Organ Body weight Ratio of rat fed with *control diet*, *Soybean*, Optimised *Cirina forda* and Non-Optimised *Cirina forda* protein supplemented diets.

APPENDIX E

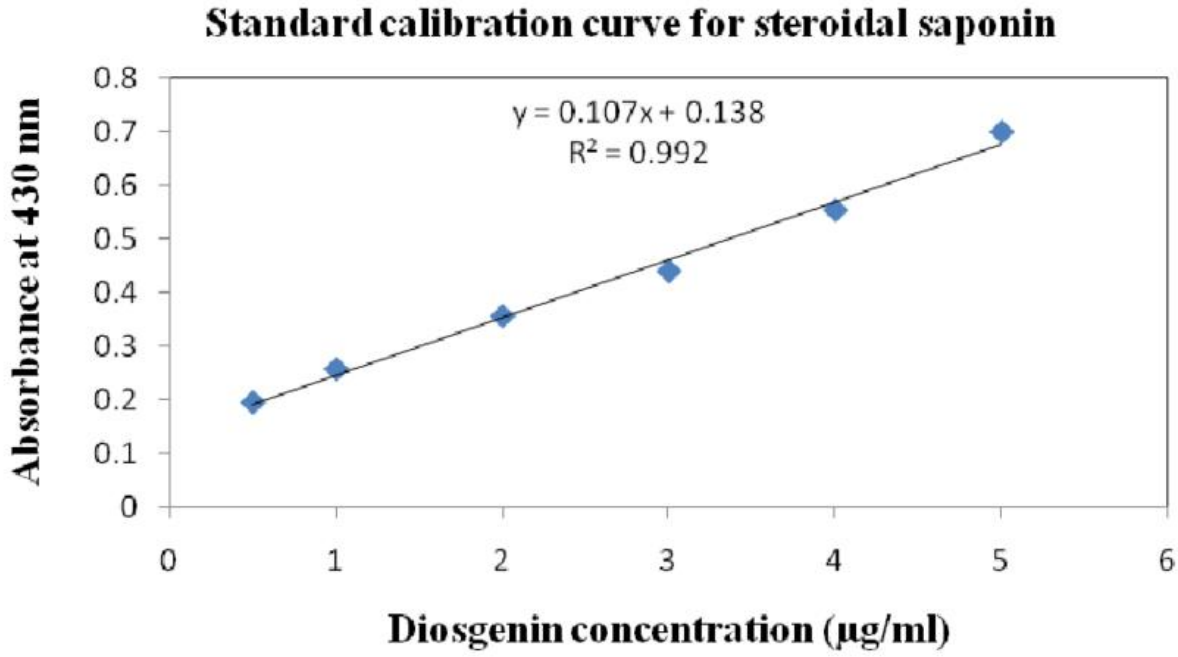


Appendix E: Urea, creatinine and electrolyte of rats fed different supplemented diet

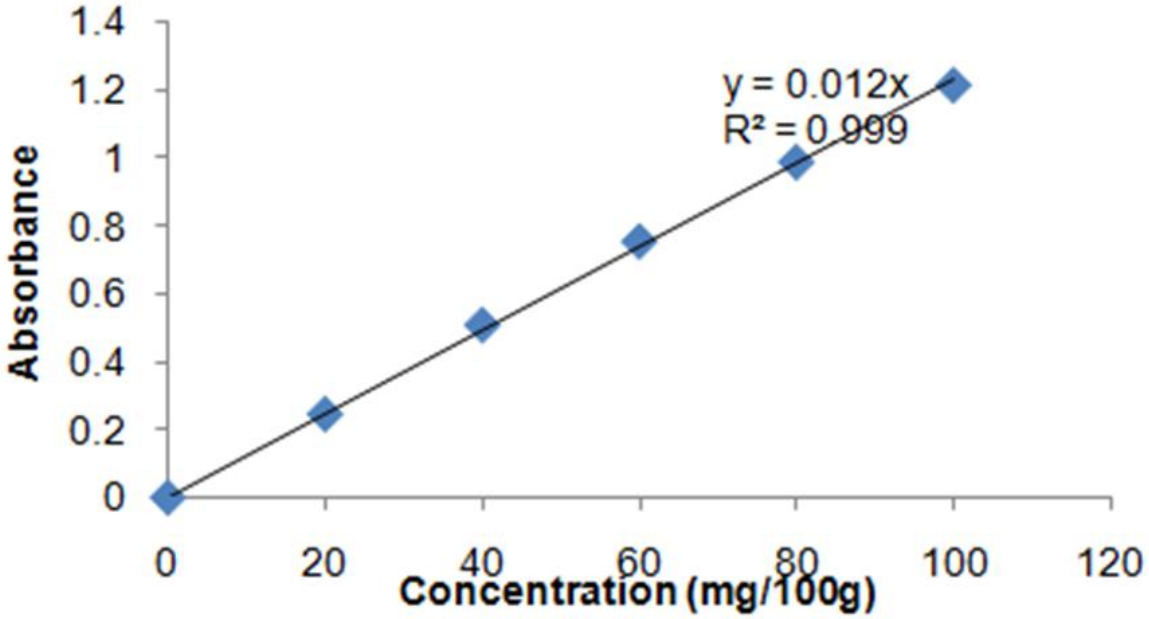
APPENDIX F



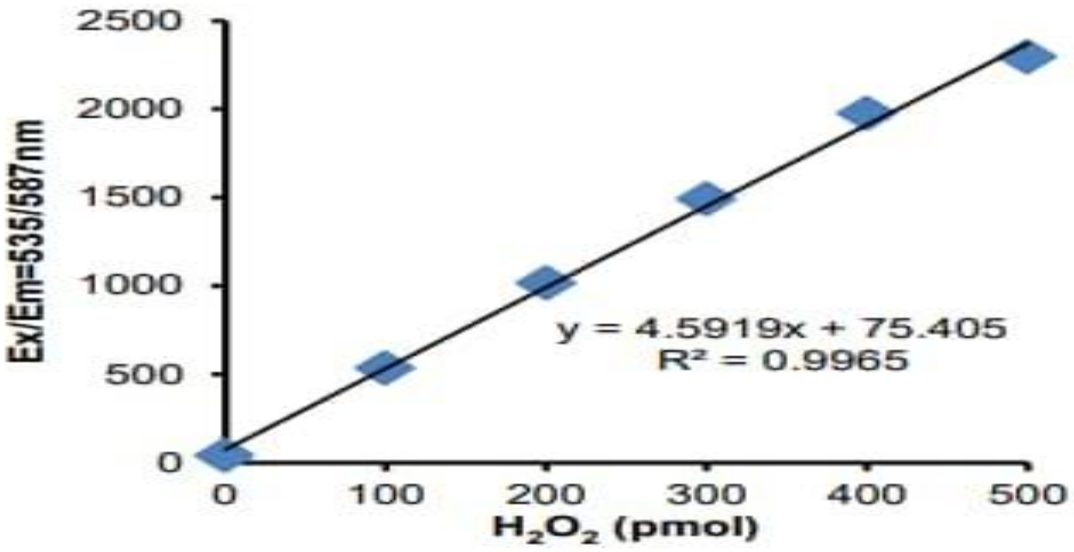
Appendpix F 1: Standard curve for Tannins



Appendix F2: Standard curve for Saponins



Appendix F3: Standard curve for alkaloids



Appendix F4: Standard curve for Oxalate

Appendix G

Appendix G1: 10 % Optimised *Cirina forda* protein

Appendix G2: 10 % Soyabean protein

Appendix G3: 10 % Non-optimised *Cirina forda* protein

Appendix G4: 20 % Soyabean protein

Appendix G5: 20 % Non- optimised *Cirina forda* protein

Appendix G6: Normal rat chow (control)