IN VITRO AND *IN VIVO* TREATMENT OF COCCIDIAL ORGANISMS IN BROILER CHICKENS USING ETHANOLIC EXTRACT OF RIPE PAWPAW (*Carica papaya*) SEEDS

BY

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MTech/SAAT/2018/7775

DEPARTMENT OF ANIMAL PRODUCTION
FEDERAL UNIVERSITY OF TECHNOLOGY
MINNA

AUGUST, 2023

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A THESIS SUMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF TECHNOLOGY IN ANIMAL PRODUCTION

AUGUST, 2023

DECLARATION

I hereby declare that this thesis **"*In vitro* and *in vivo* treatment of coccidial organisms in broiler chickens using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds"** is a collection of my original research work and it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

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…..……
SIGNATURE/DATE

CERTIFICATION

The thesis titled **"*In vitro* and *in vivo* treatment of coccidial organisms in broiler chickens using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds"** by CHRISTOPHER, Konase Samuel (MTech/SAAT/2018/7775) meets the regulations governing the award of the degree of Master of Technology (MTech) of the Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, the Creator of heaven and earth and to my lovely parents, Hon. and Mrs Christopher Ayen.

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ABSTRACT

Coccidiosis is one of the most common and expensive diseases affecting poultry in the tropics. The study was carried out to assess the anticoccidial effect of ethanolic extract of ripe pawpaw (*Carica papaya*) seed in the treatment of coccidial organisms in broiler chickens. Ripe pawpaw seeds were harvested, washed, air-dried and ground. One hundred grams (100 g) of the powder was macerated in 1.5 litres of ethanol and stirred at 3 hours intervals daily for 72 hours and then filtered using whatman paper No 3. The filtrate was concentrated by evaporating the solvent at 75^0C to obtain the extract. For *in vitro* experiment, the extract was dissolved with Dimethyl sulphoxide (DMSO) and prepared at different concentration of 20, 30, 40, and 50 mg/ml. The diluted extract was transferred into petri dishes and placed equal volume of oocysts (2ml) then, incubated at 28 – 30^0C for 24 – 30 hours. The sporozoites were counted using molasses counting chamber. The numbers of non-viable and viable sporozoites were estimated by counting the number of sporozoites in a total of 100 oocysts. For *in vivo* experiment, a total of 150 (day-old chicks) were randomly divided in five treatments (containing three replicates with 10 birds per replicate). All the treatment groups were found to be naturally infected at week 3 and laboratory examination was carried out to confirm the presence of oocysts in the faecal samples. The infected birds were administered ethanolic extract of ripe pawpaw (*Carica papaya*) seeds at 0 g (control), 2 g, 3 g, 4 g, and 5 g/litres of drinking water to T1, T2, T3, T4 and T5, respectively. Data were collected on growth performance, haematological and serum biochemical profile, egg count of the coccidial organisms in the faecal samples and histopathology of the birds were carried out. Data collected were analyzed using analysis of variance (ANOVA) at $P<0.05$ level of significance. The results of this experiments showed that the ethanolic extract of ripe pawpaw (*Carica papaya*) seeds (EERPS) contains phytochemicals such as phenols, flavonoids, saponins, alkaloids and tannins. There was lowest recovery rate at 50 mg/ml with the sporozoites recovery rate of 24.6 % in *in vitro* treatment of coccidial organisms. The experimental birds showed no significant difference ($P>0.05$) in the growth performance except in the final body weight. There was significant improvement ($P<0.05$) in the survivability of the birds and significant reduction of the oocysts in the faecal samples of the birds. There was no significant difference ($P>0.05$) in the haematological parameters except the red blood cells (RBC). There was also no significant difference ($P > 0.05$) in the serum biochemical parameters except alanine aminotransferase (ALT) and alkaline phosphatase (ALP). There was significant improvement in the histopathology of the GIT at 5g compared to other treatment groups administered ethanolic extract concentrations. Thus, ethanolic extract of ripe pawpaw (*Carica papaya*) seeds can serve as an alternative to synthetic anticoccidial drugs in the prevention and control of coccidiosis in chicken.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Coccidiosis is one of the most common disease of economic importance in poultry production systems in spite of advances in management, nutrition, chemotherapy and genetics (Lucas and Zainab, 2016). Avian coccidiosis is an infectious single-cell protozoan disease caused by gut parasites of the genus *Eimeria* (Coccidia subclass) (Gilbert *et al*., 2011). Coccidiosis caused by *Eimeria spp* invade and destroy the intestinal epithelium of poultry birds resulting in reduced feed intake, bloody diarrhoea, lesion score and reduced weight gains (Gilbert *et al*., 2011). Coccidial infection in birds is contracted through the ingestion of hardy, thick-walled sporulated oocysts which are able to survive for lengthy periods in poultry litter and in the soil. Furthermore, coccidiosis is commonly associated with poor hygiene and poor use of chemotherapeutic agents or chemical anticoccidial agents (Soulsby, 2002).

Macroscopic lesions resulting from coccidial infection in the digestive tract are predisposing factors to many gastrointestinal bacterial diseases such as clostridiosis, salmonellosis and colibacillosis. Coccidiosis infection is also aggravated by certain immunosuppressive viral diseases such as Infectious bursal disease, Marek disease and chick anaemia infectious viral disease (Lanckriet *et al*., 2010). It remains a big concern for commercial chicken production, because of the high economic costs. Various anticoccidial feed additives; most especially ionophorous antibiotics (Chapman *et al*., 2005), as well as anticoccidial drugs such as amprolium have been developed and used. The daily use and misuse of these drugs led to development of coccidial parasite drug resistance that are detrimental to consumers' health because of the presence of anticoccidial drug residues in the poultry products (Danaher *et al*., 2008). However, the

introduction of live drug-tolerant anticoccidial vaccine used on rotational basis with anticoccidial drugs is of great advantage for poultry industries in terms of effectiveness, but the vaccine in its ability to replicate in bird's intestinal tract, constitute a predisposing factor to other opportunistic disease agents (Berezin *et al*., 2010; Tewari *et al*., 2010).

Medicinal plants are considered as alternative new tools for controlling coccidiosis. For instance, the incorporation of dried leaf of creat or green chiretta (*Andrographis paniculata*) at 10, 20, 30 and 40 % in feed proved to be efficient on reduction of mortality rate of chickens against coccidial parasites (Sujikara, 2000). Among the 15 medicinal plants tested by Youn and Noh (2001), only sophora (*Sophora flavescens*) was efficient in reducing bloody diarrhoea, lesion scores and oocysts excretion. It was reported by Arczewska and Swiatkiewicz (2010) that the extracts of garlic (*Allium sativum*), sage (*Salvia officinalis*), cone flower (*Echinacea purpurea*), garden thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) have effect on coccidiosis with results comparable to the conventional anticoccidiostat in terms of live weight gain and reduction of oocysts. In the work of Okeniyi *et al*. (2007), the effective treatment of dried *C. papaya* seed extract against human intestinal parasites and without significant side effect was reported. These herbs are classified into groups of medicinal plants used by rural communities to cure many human and animal diseases (Attindéhou *et al*., 2011).

Despite the commendation of the above natural products, several challenges exist in the use of these products. For example, challenges of anticoccidial efficacy, identification of active compounds, mechanism, safety, and cost-effectiveness of plant extracts and compounds need to be addressed prior to applications. Therefore, this study is to test the anticoccidial effect of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds on broiler chickens in the treatment of coccidial organisms.

1.2 Statement of the Research Problem

The issue of drug residues have led to the ban of the use of antimicrobial agents in animal treatment in many advanced countries (Seri, 2013). Coccidiosis in birds like in other animals usually leads to extensive destruction of the intestinal epithelium resulting in reduced feed conversion efficiency, reduced body weight gains, reduction in egg production and sometimes death (Dalloul and Lillehoj, 2005).

The emergence and continuous use and misuse of anticoccidial drugs could lead to the development of coccidial drug resistance strains which could pose bio-security threat to animal and humans' health as a result of the presence of anticoccidial drug residue in poultry products such as meat and egg.

The increasing cost of coccidial drugs are now becoming major constraints to coccidiosis control in poultry farms. Though many researches have been done using plant extracts to reduce cost of production and increase productivity in order to bridge the protein gap, sources and methods of processing of some of these plant materials, and their availability makes it most difficult to be utilized by farmers.

1.3 Justification for the Study

The phytochemical compounds present in *Carica papaya* (pawpaw) have been shown to inhibit the viability of certain micro-organisms such as bacteria, protozoans and fungi (Shewangzaw and Achalew, 2016; Mathew *et al*., 2018). *Carica papaya* plant parts (leaves, seeds, latex and fruit) have been reported to possess excellent medicinal properties for the treatment of different ailments (Udo and Abba, 2018). Phytochemical analysis of *C. papaya* leaf extract revealed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenols and steroids which could be useful in the treatment of different diseases including coccidiosis (Kirtikar and Basu, 1998). Similarly, *C.*

papaya is grown widely in Nigeria and extracts derived from this plant have been reported to be safe and environmentally friendly (Udo and Abba, 2018).

Pawpaw (*Carica papaya*) seeds have been used as an alternative source in the treatment of intestinal parasites in humans (Okeniyi *et al*., 2007) and also found to exhibit anticoccidial effect on poultry chicken naturally infected with several *Eimeria species* (Dakpogan *et al*., 2018).

Natural Plants such as pawpaw (*Carica papaya*) have been reported to contain natural antimicrobial properties and could be used to replace synthetic anticoccidial drugs with high cost (Iciek *et al*., 2009).

1.4 Aim and Objectives of the Study

The aim of this study is to assess the anticoccidial effect of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds in the treatment of coccidial organisms in broiler chickens.

The objectives of the study were to;

i. examine the effect of varying levels of ethanolic extract of ripe pawpaw seeds on *in vitro* treatment of coccidial organisms;
ii. determine the weekly feed intake, weight gain, feed conversion ratio and mortality rate of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw seeds;
iii. assess the presence of oocysts in the faecal samples and litter material from broiler chickens administered varying levels of ethanolic extract of ripe pawpaw seeds;
iv. determine the haematological profile of broiler chickens administered varying levels of ethanolic extractof ripe pawpaw seeds;
v. examine the biochemical indices of broiler chickens administered varying levels of ethanolicextractof ripe pawpaw seeds; and

vi. determine the histopathology of the intestinal tracts of broiler chickens treated with varying levels of ethanolic extract of ripe pawpaw seeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of *Carica papaya* (Pawpaw) Plant

Carica papaya (pawpaw) plant is an evergreen shrub or small tree, short-lived, single-stemmed/ unbranched, hollow, herbaceous perennial with different height ranged 10 to 30 feet (Krishna *et al*., 2008). The plant is considered by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves (Anjum *et al*., 2013). It belongs to the plant kingdom, *Carica genus*, and a member of the *Caricaceae* family. It is one of the most popular and economically significant plants in the world (Ong *et al*., 2011) and genus *C. Papaya* is one of the most widely cultivated and well-known species of the four genera of the family, *Caricaceae* (Krishna *et al*., 2008). The exact region of origination of *C. papaya* is not fully documented, but it is believed that it was originated from Central America, southern Mexico and tropical America. This plant is also reported to be originated in Costa Rica and distributed as a plantation crop in India, Sri Lanka, Hawaii, Australia, and in tropical and subtropical regions (Krishna *et al*., 2008). *Carica Papaya* fruit is readily available throughout the year and commonly consumed fresh as a dessert or juice (Ameen *et al*., 2012). *C. Papaya* seeds are black in color and embedded in the fruit pulp (Kadiri *et al*., 2016). Generally, ripe *C. papaya* seeds represent about 16 % of the fresh fruit weight (Sugiharto, 2020). The herbaceous perennial plant can also be known as *C. Papaya* melon tree, Pawpaw or papau, Kapaya, Lapaya, Papyas, Papye, Tapayas, and Fan mu gua. The entire *C. papaya* plant possesses various phytonutrients and can be considered for commercial, industrialand pharmaceutical purposes. The *C. papaya* plant is best with a large variety of phytonutrients and antioxidant, antimicrobial, and anti-dengue properties (Abd Elgadir *et al*., 2014).

2.2 Botanical Classification

Domain: Flowering plant

Kingdom: *Plantae*

Sub Kingdom: *Tracheobionta*

Class: *Magnoliopsida*

Subclass: *Dilleniidae*

Superdivision: *Spermatophyta*

Phyllum: *Steptophyta*

Order: *Brassicales*

Family: *Caricaceae*

Genus: *Carica*

Botanical Name: *Carica papaya*

2.3 Botanical Description of *Carica papaya* (Pawpaw)

2.3.1 Pawpaw plant

Carica papaya plant is a large, single–stemmed herbaceous perennial tree having 10 - 30 feet height. The leaves are very large (up to 2½ feet wide), having webbed appendages or deeply incised with entire margins and petioles of 1-3 feet in length. Stems are hollow, light green to tan brown in color with diameter of 8 inches and bear prominent of scars (Arvind *et al*., 2013).

2.3.2 Pawpaw fruit

Carica papaya fruits are big oval in shape and sometimes called pepo-like berries, since they resemble melon by having a central seed cavity. Fruits are borne axillary on the main stem, usually singly but sometimes in small clusters. Fruits weigh from 0.5 up to 20 lbs, and are green when unripe, turning yellow or red orange when ripe. Flesh is yellow-

orange to pinkish orange at maturity. The edible portion is surrounded by large central seed cavity. Individual fruits mature in 5 - 9 months, depending on cultivator and temperature. Plants begin bearing fruits in 6 - 12 months (Arvind *et al*., 2013).

2.3.3 Pawpaw flowers

Carica papaya plants are dioecious or hermaphroditic, producing only male, female or bisexual (hermaphroditic) flowers. *Carica Papaya* are sometimes said to be "trioecious" meaning that separate plants bear either male, female, or bisexual flowers. Female and bisexual flowers are waxy, ivory white, and borne on short penducles in leaf axils, along the main stem. Flowers are solitary or small cymes of 3 individuals. Ovary position is superior. Prior to opening, bisexual flowers are tubular, while female flowers are pear shaped. The bisexual plants produce the most desirable fruits and are self-pollinating, therefore are preferred over female or male plants. Female *C. papaya* flowers are distinguished by pear shaped, when unopened. A male *C. papaya* have smaller flowers borne on long stalks whereas, bisexual flowers are cylindrical (Arvind *et al*., 2013).

2.4 Chemical Composition (Active Components) of *Carica papaya* (Pawpaw)

Carica Papaya contains valuable constituents in different parts of the plants such as fruits, leaves, and seeds with different proportions. The Phytochemical analysis of the leaves showed that the leaves hold saponins, cardiac glycosides, and alkaloids whereas tannin was absent (Ayoola and Adeyeye, 2010). Another study revealed that the leaves of *C. Papaya* contained phenolic acids as the main compound, whereas chlorogenic acid was found in trace amounts, compared to the flavonoids and coumarin compounds (Canini *et al*., 2007). The fruits are excellent sources of vitamins (such as A and C), flavonoids, and various other minerals (such as calcium) with different amount according to maturation stages (Bari *et al*., 2006). Chemical constituents present are different in red fleshed and

yellow fleshed *C. papaya*. Earlier report confirmed the presence of total lycopene content of red fleshed *C. papaya* fruit was notably higher than that of yellow fleshed fruit (Schweiggert *et al*., 2011). *C. papaya* seed is reported to contain important types of chemical constituents and such ingredients shows role in the health prevention and treatment. Earlier study confirmed that seeds of *C. papaya* are excellent sources of proteins, lipids, and crude fibre and contained appreciable quantities of calcium and phosphorus. Similarly, the presence of toxicants, such as glucosinolates, was also reported (Marfo *et al*., 1986).

2.5 Therapeutic Role of *Carica papaya* (Pawpaw)

Carica papaya has medicinal value in the health management due to it rich source of vitamins, carbohydrate and essential minerals in different parts of the plants. Literature reported that *C. papaya* parts such as seeds, fruits, and leaves has therapeutic importance in the diseases cure as presented below;

2.5.1 Antioxidant activity

Natural products or plant products are a good source of antioxidant and show vital role in the diseases cure and prevention. A study has shown that *C. papaya* seeds aqueous extract has powerful antioxidant activity in H_2O_2 oxidative stress induced human skin. Detroit 550 fibroblasts and additional finding also confirmed that the extract is not toxic, decreases cell death, ensures Ca_{2+}homeostasis, and counteracts mitochondrial dysfunctionality (Panzarini *et al.,* 2014). Similarly, another study was performed to evaluate the antioxidant and cytotoxic potential of extracts of fruits and seeds and the study results proved that both ethyl acetate fractions from the fruits and seeds of *C. Papaya* are high in antioxidant activities with IC50 values of 30.61 µg/ml and 25.97 µg/ml and cytotoxic with IC50 Wound healing effect of 163.96 µg/ml and 142.27 µg/ml

respectively (Khor and Wong, 2014). *C. Papaya* leaf (CPL) aqueous extract effects on alcohol-induced acute gastric damage was analyzed and finding of the study revealed that the gastric ulcer index was significantly reduced in rats treated with CPL extract as compared with alcohol treated controls. Additionally, the result also confirmed that CPL extract showed some protection with a reduction in plasma lipid peroxidation level and increased erythrocyte glutathione peroxidase activity (Indran *et al*., 2008).

2.5.2 Anti-inflammatory activity

Numerous plant products or isolated plant derivatives shows role as anti-inflammatory via modulation of various activities. Drugs currently used such as nonsteroidal anti-inflammatory drugs (NSAIDs) produce intestinal tract ulcers and other complications in the stomach. A study confirmed anti-inflammatory activity of *C. papaya* seeds and methanolic extract showed inhibition ranging from 57.1 % to 64.2 % is lower than 85.7 % of aspirin, a standard anti-inflammatory drug (Amazu *et al*., 2010).

2.5.3 Analgetic activity

Numerous natural products from plant source showed analgetic effect without any complications. In view of this, *C. papaya* also shows an important role in analgetic activity. A study was carried out to investigate the analgetic activity of *C. papaya* leaves extracts in mice model using acetic acid-induced pain and findings revealed that all extracts such as n-hexane, ethyl acetate and ethanol at 0.175, 0.35 and 0.70 mg/kg doses showed significant analgesic activity as compared to control group (Hasimun *et al*., 2014).

2.5.4 Wound healing effect

Numerous plants and their constituents showed important role in wound healing. A study was conducted to evaluate the aqueous extract of *C. papaya* fruit for its wound healing

ability in streptozotocin-induced diabetic rats using excision, and dead space wound models and finding revealed that extract-treated animals exhibited a 77 % reduction in the wound area as compared to controls that were 59 % (Nayak *et al*., 2007). Similarly, another study was carried out to evaluate the wound healing potential of aqueous extract of *C. papaya* roots and finding confirmed that latex treated animal's exhibit 89.40 % reduction in wound area as compared to controls which were 80.38 % and furthermore extract-treated wounds are found in epithelial faster as compared to controls (Tiwari *et al*., 2011). Another study also confirmed that *C. papaya* leaf aqueous extract has health benefits effect as wound healing activity in rats (Mahmood *et al*., 2005).

2.5.5 Antimicrobial activity

Currently, treatment in health management based on antibiotic is effective, but also causes antibiotic resistance. In view of this, medicinal plants show effective role in the control of bacterial growth. A study was carried out on *C. papaya* root extracts using water and organic solvents to evaluate the antibacterial activity against some pathogenic bacteria and result revealed that aqueous extracts did not show significant activity, whereas organic extracts showed significant activity with the methanol extracts demonstrating the highest activity against the test bacteria. In addition, extracts demonstrated higher activities against all the gram-negative bacteria than the gram-positive bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Salmonella typhi* (Doughari *et al*., 2007). Similarly, another study was performed to evaluate the antibacterial activity of aqueous chloroform extract of leaves and aqueous methanolic extract of seeds of *C. papaya* and finding revealed that aqueous and methanolic extract of seeds were effective to inhibit the bacterial pathogens, whereas the chloroform extract of the leaves did not show any inhibition against the bacteria and the aqueous leaf extract was powerful in inhibition of bacterial pathogens (Peter *et al*., 2014). Antibacterial

activity of extracts of *C. Papaya* fruit was evaluated using isolates from wound culture and results showed that extracts established antibacterial activity and this was more prominent with alcohol extracts than that of water (Akujobi *et al.*, 2010).

2.5.6 Gastro-protective effect

Numerous agents such as food ingredients, microorganism and drugs are one of the main challenges in gastric ulcer/ complications. Plant products show anti-ulcer effect, but the exact mechanism is not understood completely. A study was carried out to evaluate the gastro protective effects of aqueous *C. papaya* seed extract on ethanol induced gastric ulcer in male rats and finding revealed that the extract protected the gastric mucosa against ethanol effect and extract significantly reduced the gastric juice volume and gastric acidity in a dose dependent manner when compared with the control (Abisola and Wahab, 2012). Similarly, another study to evaluate the anti-ulcerogenic activities of *C. Papaya* extract on aspirin–induced ulcer in rats was reported, and the result revealed that *C. Papaya* exert its gastro protective effect via free radical scavenging action (Ologundudu and Lawal, 2008).

2.5.7 Hepato-protective effect

A study was performed to evaluate the hepato-protective effects of *C. Papaya* against carbon tetrachloride (CCl_4) induced hepatotoxicity and compared it with that of vitamin E and results confirmed that *C. Papaya* and vitamin E showed significant hepato-protection against CCl_4. Similarly, another findings confirmed that pretreatment with medium and high doses of C. *Papaya* induced hepatotoxicity, but *C. Papaya* showed more significant changes in ALP level than vitamin E (Sadeque *et al.*, 2012). The experiment was made to examine the hepato-protective effect of *C. papaya* leaves against ethanol, and anti-tubercular drug-induced liver damage and results revealed that hepato-

protective activity was evident by the significant reduction in the levels of all serum markers in both models (Pandit *et al*., 2013). Another study was performed to examine the antihepatotoxic activity of ethanol and aqueous extracts of *C. Papaya* and finding confirmed that ethanol and aqueous extracts of *C. Papaya* showed remarkable hepato-protective activity against CCl_4 induced hepatotoxicity (Rajkapoor *et al*., 2002).

2.5.8 Anti-ulcerogenic effect

A study was carried out to evaluate the anti-ulcerogenic and antioxidant activities of aqueous extract of *C. papaya* seeds against indomethacin-induced peptic ulcer in male rats and findings revealed that there is significant increase in gastric pH and percentage of ulcer inhibition treated with *C. papaya* seeds extract compared to indomethacin-induced ulcer rats (Oloyede *et al*., 2015).

2.5.9 Anti-tumor (cancerous) activity

Constituent of plants shows therapeutic role in cancer prevention and treatment (Rahmani *et al*., 2014). In view of this, *C. papaya* and their valuable constituents have a significant role in the cancer management. A study was carried out to evaluate the effect of aqueous extract of *C. papaya* leaves on the growth of various tumor cell lines and on the anti-tumor effect of human lymphocytes and results showed that growth inhibitory activity of the *C. papaya* extract on tumor cell lines derived from cervical carcinoma (Hela), breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HepG2), lung adenocarcinoma (PC14), pancreatic epithelioid carcinoma (Panc-1), and mesothelioma (H2452) in a dose-dependent manner. In addition, *C. papaya* extract inhibited the proliferative responses of haematopoietic cell lines, including T-cell lymphoma (Jurkat), plasma cell leukemia (ARH77), Burkitt's lymphoma a (Raji), and anaplastic large cell lymphoma (Karpas-299) (Otsuki *et al*., 2010). Furthermore, men consuming lycopene-rich fruits such as *C.*

papaya, are 82 % less likely to have prostate cancer compared to those consuming the least lycopene-rich foods (Mehul and Samir, 2016).

2.5.10 Anti-diabetic (hypoglycemic) activity

Diabetes mellitus and its linked complication are major health problem worldwide. Oral hypoglycemic drugs are effective and useful in the treatment but shows adverse complications. Natural products show an important role in the management of diabetes mellitus and complications. A study was performed to evaluate the antihyperglycemic and hypolipidemic activity of aqueous extract of leaves of *C. papaya* and the result revealed that extracts showed a significant reduction in blood glucose level and serum lipid profile levels with 400 mg/ kg body weight in alloxan-induced diabetic rats when compared with the control (Maniyar and Bhixavatimat, 2012). Another study results revealed that aqueous extract of *C. papaya* with 0.75 g and 1.5 g/ 100 ml significantly decreased blood glucose levels in diabetic rats and also decreased aminotransferases, triacylglycerol and cholesterol blood levels (Juárez-Rojop *et al*., 2009).

2.5.11 Anti-nephrotoxicity effect

An experiment was performed to evaluate the nephro protective role of ethanolic extract of the *C. papaya* seeds and pumpkin seeds and the results confirmed that ethanolic extract of both sides showed protection against cisplatin-induced nephrotoxicity and antioxidant studies such as nitric oxide scavenging activity. Lipid peroxidation in kidney also supported the nephro protective activity of both seeds (Debnath *et al*., 2010). Similarly, another study was performed to evaluate the protective effect of aqueous extract of *C. Papaya* seeds on gentamicin-induced hepatotoxicity and nephrotoxicity, and the result of

the findings revealed that the administration of aqueous extract before gentamicin exposure prevented severe alterations of biochemical parameters and disruptions of liver and kidney structures (Nale *et al*., 2012).

2.5.12 Diuretic effect

A vital study revealed that extracts of *C. papaya* root, when given orally to rats at a concentration of 10 mg/kg, confirmed significantly increased urine output, which was 74 %, of the effect of an equivalent dose of hydrochlorothiazide (Sripanidkulchai *et al*., 2001).

2.5.13 Anti-malaria effect

Human malaria is one of the major health problems worldwide. However, safe and effective mode of treatment is needed to control malaria and its complications. To this regard, numerous medicinal plants have confirmed their role in the control of malaria. A study confirmed that concentrations at 25, 50, 100 and 150 μg/ml of ethanol leaf extracts of *C. papaya* exhibited promising inhibitory activity against the CQ-sensitive strain with IC 50 values at 40.75, 36.54, 25.30 and 18.0 % and in CQ-resistant at 50.23, 32.50, 21.45 and 23.12 % against *P. falciparum* (Kovendan *et al*., 2012). Another study was conducted to evaluate the platelet increasing property of *C. papaya* leaves juice in patients with dengue fever and which is a randomized controlled trial of 228 patients that confirmed a significant increase in platelet count and dengue hemorrhagic fever after administration of *C. papaya* leaf juice (Subenthiran *et al*., 2013). Similarly, another study reported the positive role of *C. papaya* leaves in the treatment of dengue and results revealed that thrombocyte count increased from 28000/ micro liter to 138000/ microlitre after administration of *C. papaya* leaves at the end of five days (Siddique *et al*., 2014).

2.5.14 Antifertility effect

A study results confirmed that oral administration of the aqueous extract of *C. papaya* seeds at 50, 100 and 800 mg/kg body weight altered the normal sequence of estrous cycle, but showed no effect on ovulation and the number of ova shed (Dosumu *et al*., 2008). Another finding reported that cholesterol levels in testes were notably decreased by the *C. papaya* seeds extraction indicating decreased mobilization towards androgenesis and in this manner promotes inhibition of spermatogenesis in the testis (Lakshman and Changamma, 2012). Similarly, another study results revealed that chloroform extract of *C. papaya* seeds and other *C. papaya* products have been used in disease treatment worldwide. Numerous studies based on *in vivo* leads to azoospermia without adverse toxicity after 90 days of treatment in langur monkeys and sperm functional tests confirmed the voided spermatozoa after 30 and 60 days of treatment were in the infertile range (Lohiya *et al*., 2002).

2.5.15 Anti-amoebic activity

A vital experiment was carried out to evaluate the anti-amoebic activity of methanol extract of mature seeds of *C. papaya* based on *in vitro* on axenic culture of entamoeba histolytic and results revealed that methanol extract of *C. papaya* of seed was > 62.5 µg/ml as compared to < 0.8 µg/ml for metronidazole (Sarker *et al*., 2010).

2.5.16 Anxiolytic and sedative effects

Medicinal plants and their constituents also shows role as anxiolytic and sedative effects. A study was conducted to evaluate the anxiolytic and sedative effects of ethanolic *C. papaya* pulp extract in mice, and the results revealed that extract at 100 mg/kg showed anxiolytic effect (Athesh *et al*., 2012).

2.5.17 Anti-obesity effect

Numerous plants and their products, including *C. papaya* has confirmed the anti-obesity effect. A study was conducted to assess the anti-obesity potential of aqueous extract of *C. papaya* fruit on high-fat cafeteria diet (HFD) fed obese rats and result revealed that the body weight, organ weight of the liver, kidney and spleen were significantly decreased in the treated groups than in the HFD control group animals. In addition, Findings of the study also confirmed that serum Glucose, Triglycerides, Total cholesterol, LDL-Cholesterol, and VLDL-Cholesterol were significantly decreased, whereas HDL-Cholesterol was elevated in the treated groups in a dose-dependent manner as compared to the HFD group (Kebebew and Shibeshi, 2013).

2.5.18 Rheumatoid arthritis

Vitamin C-rich foods, such as *C. papaya*, provide humans with protection against inflammatory poly-arthritis, a form of rheumatoid arthritis involving two or more joints (Mehul and Samir, 2016).

2.5.19 Promote lung health

For people that smoke, or frequently exposed to second hand smoke. Eating vitamin A-rich foods such as *C. papaya*, will help the lung stay healthy and save life (Mehul and Samir, 2016).

2.6 Safety and Toxicities of *Carica papaya* (Pawpaw)

Various medicinal plants and their constituents show role in disease prevention and treatment at certain doses. Overdose or improper dose causes complications and alters various biological activities. However, safe dose of any plants or products is very important in the health management. A study was carried out to investigate the toxicity of crude protein leaf extracts on Sprague-Dawley rats and result findings revealed that *C.*

papaya leaf juice did not show any toxicity effect (Halim *et al*., 2011). Similarly, another study was performed about acute and chronic oral toxicity study on the aqueous and ethanol leaf extracts of *C. papaya* in Wistar rats and result revealed that no deaths or signs of acute oral toxicity were reported. Additionally, oral sub-acute and sub-chronic toxicity included hypoglycemia, hypolipidemia, and hyperglycemia, increased AST values in aqueous and ethanol extract experiments, respectively (Tarkang *et al*., 2012).

2.7 Nutritional Value of *Carica papaya* (Pawpaw) Plant

Carica papaya is a common fruit, which has a high nutritive value, low in calories, rich in natural vitamins, and minerals and reasonably priced. The comparative low calories content (32 Kcal/ 100 g of ripe fruit) makes the fruit favoured by obese people who are into weight reducing regime. *Carica. papaya* has low carotene compared to other fruits such as apples, guava and plantains, which helps to prevent damage by free radicals. Unripe green *C. papaya* is used as vegetable and does not contain carotene but all other nutrients are present. The fruit is a rich source for different types of enzymes such as papain. Vegetable pepsin present in good amount in unripe fruit is an excellent aid to digestion, which helps to digest the protein in food at acid, alkaline and neutral medium. The crude papain present in *C. papaya* can help celiac disease patients who cannot digest the wheat protein gliandin. *C. papaya* has the ability of tenderizing meat, as this knowledge is being put to use by cooking meat with raw *C. papaya* to make it tender and digestible (Marotta *et al*., 2006). The fermented *C. papaya* fruit is a promising antioxidant. It improves the antioxidant defense in elderly patients even without any overt antioxidant deficiency state at a concentration of 9 g/ day administered orally. The *C. papaya* lipase, a hydrolase enzyme tightly bonded to the water insoluble fraction of crude papain, is considered as a "naturally immobilized" biocatalyst (Marotta *et al*., 2006). *C. papaya* noticeably increases iron (Fe) absorption from rice meal, which was measured in

parous Indian women, using the erythrocyte utilization of radioactive Fe method. The *C. papaya* black seeds are edible and have a sharp, spicy taste. They can be ground up and used as a substitute for black pepper. In some parts of Asia, the young leaves of *C. papaya* are steamed and eaten like spinach (Marotta *et al*., 2006).

2.8 Nutritional Compositions of *Carica papaya* (Pawpaw) Seeds

The use of agro-industrial by-products in livestock rations has been encouraged due to the continuous increase in price of conventional feedstuffs. The agro-industrial by-products such as *C. papaya* seeds has been explored extensively as potential feed ingredients for livestock such as poultry. The seeds of *C. papaya* contain an adequate amounts of crude protein, fat, and ash that can be efficiently be utilized by the chickens. *C. papaya* seed also contains substantial amount of minerals that is essential for the metabolism, body functions and health of poultry (Moses and Olanrewaju, 2018). *C. papaya* seed contains Ca (681), Mg (424), P (2,116), Fe (5.80) and Na (23.4) mg/ 100 g (Maisarah *et al*., 2014). Similarly, Moses and Olanrewaju (2018) also reported that *C. papaya* seeds contains Ca (6.43), K (721), Fe (4.20), and Zn (6.41) mg/ 100 g. Adesuyi and Ipinmoroti (2011) documented that *C. papaya* seeds contains Na, K, and Ca ranging from 33.6-16.2, 47.7- 17.0, and 2.52-4.14 mg/ 100 g, respectively. *C. Papaya* seeds also contains Mg, Zn, and Fe ranging from 0.53- 2.81, 1.26-2.88, and 0.39-1.47 mg/ 100 g respectively. The minerals Mn, Cu, Pb, and P were also found in *C. papaya* seeds ranging from 1.11-1.27, 0.05-0.19, 0.00010-0.00013 and 28.5-58.6 mg/ 100 g, respectively. The variations in the mineral content across the papaya seeds could be as a result in varieties or cultivars, ripening stages, soil conditions (where the *C. papaya* plants are cultivated) and climatic conditions (Dos Santos *et al*., 2014).

In general, plant-derived products are rich in vitamins, including papaya seeds. Maisarah *et al*. (2014) reported that *C. papaya* seed contains ascorbic acid 14.4 mg/ 100 g, β-

carotene 120 µg/ 100 g, and vitamin E 4.09 mg/ 100 g. In addition, ripe *C. papaya* seeds contained vitamin A 135 IU/ mg, vitamin C 14.7 IU/ mg, riboflavin 0.02 mg, thiamine 0.03 mg, and niacin 0.11 mg (Chukwuka *et al*., 2013). However, the contents of vitamins vary with the ripening stages of the *C. papaya* fruits. In the unripe condition, *C. papaya* seed contains vitamin A 87.2 IU/ mg, vitamin C 11.7 IU/ mg, riboflavin 0.01 mg, thiamine 0.05 mg, and niacin 0.10 mg (Chukwuka *et al*., 2013). On this note, *C. papaya* seed seem to be more beneficial for livestock production including poultry, due to its high nutrient content.

2.9 Anti-Nutritional Factors of *Carica papaya* (Pawpaw) Seeds

Carica papaya seed contains some anti-nutrient factors that may inhibit the absorption and utilization of nutrients including minerals by poultry. These anti-nutrient factors include oxalate, tannins, and phytate (Adesuyi and Ipinmoroti, 2011). El-Safy *et al*. (2012) reported that *C. papaya* seeds contained phytic acid, tannins, and oxalate at 23.3, 10.6, 1.89 mg/ 100 g respectively. The *C. papaya* seed also contains trypsin inhibitors at 1.77 TIU/ mg. Generally, the quantities of the anti-nutrient factors are relatively low, but the quantities may vary among the cultivars of *C. papaya* seeds. For safety reason, the determination of anti-nutritional factors is of great importance before using *C. papaya* seeds in animal's production.

2.10 Growth Promoting Effect of *Carica papaya* (Pawpaw) Seeds

In the post-antibiotic era, the presence of natural growth promoters for livestock is highly essential. Therefore, the withdrawal of antibiotics growth promoters from diets has been attributed to the retarded growth rate and thus poor economic performance of livestock including poultry farming. Though, findings are still limited, it was reported that papaya seeds can improve the production performances of poultry, including increasing growth

rate, egg production, and feed efficiency of poultry. In broiler chickens, the incorporation of 1 % *C. papaya* seed powder in rations increased the final body weight and feed intake (Muazu and Aliyu-Paiko, 2020). Banjoko *et al*. (2020) reported that varying levels of *Carica papaya* leaf as an anticoccidial for broiler chickens at the rate of 200, 400 and 600 g showed effectiveness of *C. papaya* leaf on the final body weight. Similarly, Rachmatika and Prijono (2015) also reported that the incorporation of 1.2 % *C. papaya* seeds in diets increased body weight gain, reduced feed intake, and improved feed efficiency of Raja ducks. Furthermore, the inclusion of 0.5, 1.0, and 2 % *C. papaya* seeds in the rations increased daily weight gain of pullet when compared with the control (Nideou *et al*., 2017). In accordance with the growth-promoting effect, the administration of 0.5 % papaya seed meal in diets increased egg weight, egg production, and feed conversion ratio of broiler breeder and improved day-old weight of chicks (Nideou *et al*., 2020). In addition, Nghonjuyi *et al*. (2020) also reported that the administration of *C. papaya* seeds extract at the levels of 480, 960, and 3200 mg/kg body weight increased body weight of Kabir chicks. The definite mechanism by which *C. papaya* seeds improved the growth performance of poultry remains unclear, but Muazu and Aliyu-Paiko (2020) suggested that the improvement in organoleptic characteristics of the feed due to *C. papaya* seed administration seemed to increase feed intake and thereby growth performance of poultry. Furthermore, the improvement of gastrointestinal conditions and thus increased the digestibility and utilization of nutrients seemed to improve the production performance in poultry (Nideou *et al*., 2017). In addition, the antimicrobial, anthelmintic and antiparasitic activities of *C. papaya* seeds were most likely to improve the gastrointestinal ecology and thus improve the health conditions and growth performance of poultry (Ameen *et al*., 2012 and Dakpogan *et al*., 2019). The contents of antioxidants (Kadiri *et al*., 2016) and minerals (Moses and Olanrewaju, 2018) in *C. papaya* seeds seemed also

to be responsible for alleviating the negative impact of stress and also improving the health of poultry. Nghonjuyi *et al*. (2020) further suggested that the presence of papain, chymopapain, caricain, and glycyl endopeptidase may improve the digestive process and thus enhance the growth rate of chickens. In the molecular level, *C. papaya* seeds extract has also been reported to increase cell proliferation and reduce the apoptotic cells (Dosumu *et al*., 2003).

2.11 Antimicrobial, Antihelminthic and Antiparasitic Activities of *Carica papaya* (Pawpaw) Seeds

Generally, seeds of tropical fruits are rich in phytochemicals that may be essential to control and modulate the population of pathogens in the gastrointestinal tract of animals. In view of this, *C. papaya* seeds from such fruits have been reported to contain alkaloids, flavonoids, steroids, saponins, papain, and terpenoids possessing antimicrobial, antihelminthic as well as antiparasitic activities (Masfufatun *et al*., 2019 and Hidayati *et al*., 2019). Several studies have reported the antimicrobial activities of *C. papaya* seeds. For instance, Muhamad *et al*. (2017) reported the antibacterial properties of *C. papaya* seeds extract against *Salmonella enteritidis*, *Vibrio vulnificus*, *Proteus mirabilis*, and *Bacillus cereus*. Similarly, Peter *et al*. (2014) showed the antibacterial activities of *C. papaya* seeds toward *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella typhi*. Furthermore, Hidayati *et al*. (2019) reported the antibacterial activity of *C. papaya* seeds against *E. coli* and *S. typhi*, while Masfufatun *et al*. (2019) demonstrated the efficacy of *C. papaya* seeds against *Vibrio cholerae* and opportunistic pathogenic yeast *Candida albicans*. To this regard, study also showed the antifungal activity of *C. papaya* seeds toward *Aspergillus flavus*, *C. albicans,* and *Penicillium citrinum* (Singh and Ali, 2011). It is clear that *C. papaya* seeds could serve as antiparasitic agents and may replace the use of synthetic antiparasitic drugs in poultry production. The

presence of papain in *C. papaya* seeds seemed to be responsible for reducing the number of parasites in the gastrointestinal tract of poultry as papain was able to digest bacteria and parasitic cells (Ameen *et al*., 2012). Furthermore, the anthelmintic or antiparasitic properties of *C. papaya* seeds are attributed to the presence of carpaine and carpasemine that have wide spectrum antibacterial and antiparasitic activities (Kumar and Devi, 2017). Udo and Abba (2018) reported the *in vitro* anticoccidial efficacy *of Allium sativum* and *Carica papaya* extract at a concentration of 2.5g, 5.0 g and 10 g/l of distilled water when 4800 unsporulated but viable oocysts were inoculated at room temperature in the laboratory. Highest efficacy at 48 hours with the highest number of unsporulated oocysts was seen at concentration 10 g at 68 % yield for aqueous *Carica papaya* and at 65 % for powder *Carica papaya*. The anthelmintic effect of pawpaw (*Carica papaya*) led to complete mortality of *Ichthyophthirius multifilis* with *in vitro* treatment of the extract in fish (Ekanem *et al*., 2004). In addition, Nideou *et al*. (2017) also pointed out the presence benzyl isothiocyanate in *C. seeds* which may inhibit energy metabolism and motility of parasites, while papain destroys the cuticle of parasites. The presence of benzyl isothiocyanate in *C. papaya* seeds may also be responsible for the mitochondrial dysfunction of parasites (Zhang and Chen, 2017). The antimicrobial, anthelmintic, and antiparasitic activities of the papaya seeds were most likely to improve the gastrointestinal ecology and thus improve the health conditions and growth performance of poultry (Kadiri *et al*., 2016). These conditions may result in the reduction of parasites in the gut of chickens. Furthermore, Jiménez-Coello *et al*. (2013) also reported that fatty acid in the *C. papaya* seeds may also decrease the number of parasites from parasite stages, blood trypomastigote, and amastigote (intracellular stage). The daily administration of *C. papaya* seeds may prevent parasite eggs from growing and reaching mature stage in the gastrointestinal tract of chickens. Generally, the antiparasitic efficacy of *C. papaya* seeds

on poultry are dose-dependent, in which higher level of *C. papaya* seeds results in more reduced egg worms in the excreta (Nideou *et al*., 2017). However, high level of *C. papaya* seeds should be noted, as it may negatively affect nutrient absorption and retention and thus production performance of poultry (Bolu *et al*., 2009).

2.12 Antioxidative Activity of *Carica papaya* (Pawpaw) Seeds

Generally, plants are known as good natural antioxidant sources that may be useful for livestock such as poultry. *C. Papaya* seeds have long been explored for its potential as natural source of antioxidants. *C. papaya* seeds have been reported to contain substantial amount of phenolic compounds such as ferulic acid, caffeic acid, p-coumaric acid, kaempferol-3-glucoside, p-hydroxybenzoic acid, and quercetin-3-galactoside (Kadiri *et al*., 2017). Furthermore, *C. papaya* seed are rich in polyphenols, flavonoids, alkaloids, tannins, and saponins (Salla *et al*., 2016) and also contains lycopene, which is highly reactive against free radicals. In addition, *C. papaya* seeds are also rich in vitamin C, vitamin E, and β-carotene, which are good antioxidants (Muazu and Aliyu-Paiko, 2020 and Maisarah *et al*., 2014). The antioxidative properties of *C. papaya* seeds have also been tested *in vitro* experiment, in which *C. papaya* seeds could protect the HepG2 cells from oxidative stress (Salla *et al*., 2016). To this regard, Panzarini *et al*. (2014) reported that *C. papaya* seeds extract served as a potent free radical scavenger and protected the Detroit 550 fibroblasts undergoing H_2O_2 oxidative stress. Currently, poultry nutritionists are getting interested in finding the natural antioxidants as the alternative to chemical-based antioxidants, as the use of chemical-based antioxidants is inducing a negative effect on humans as the consumers of poultry products (Sugiharto *et al*. 2017). In view of this, *C. papaya* seed powder at 0.5 and 1.0 % levels resulted in increased serum antioxidant activity, superoxide dismutase (SOD) and catalase (CAT) and decreased lipid peroxidation in broiler chickens (Muazu and Aliyu-Paiko, 2020). Furthermore, *C. papaya*

seeds extract increased the plasma level of SOD and decreased malondialdehyde (MDA) concentration, indicating the improvement of the antioxidative status of mice (Sandhiutami *et al*., 2016). Similarly, Venkateshwarlu *et al*. (2018) documented the increased SOD, CAT, and glutathione and decreased MDA levels in the serum of rats with the administration of 200 and 400 mg/kg of *C. papaya* seeds extract. In addition, *C. papaya* seeds also seemed to inhibit the generation of superoxide (free radicals) in poultry, as a result of the presence of isothiocyanate (Nakamura *et al*., 2007). It is much likely that the antioxidative components in *C. papaya* seeds are responsible for scavenging the free radicals (reactive oxygen molecules) and thus protecting the poultry from antioxidative stress. The antioxidants and minerals properties in *C. papaya* seed seemed also to be responsible for alleviating the negative impact of stress and improving the health of poultry (Moses and Olanrewaju, 2018).

2.13 Haematological Activity of *Carica papaya* (Pawpaw)

Haematological parameters still remains a good indicator for the animal's physiological status and as a result there is a positive correlation with the animal's health and nutritional status (Adejumo, 2004). *Carica papaya* (pawpaw) is reported to improve the haematological and immune profile, that is, increased packed cell volume, red blood cells, haemoglobin concentration, and lymphocyte counts (Ameen *et al*., 2012). This is likely attributed to the chemical and nutritional components found in pawpaw and it parts. Bolu *et al*. (2009) reported hematological values such as RBC, PCV and Hb of broiler chickens were significantly affected by inclusion of dried pawpaw seeds at the rate of 5, 10 and 15 %. Agboola *et al*. (2018) reported highest values in RBC, PCV and Hb when pawpaw leaf meal was fed to broiler chicken under normal and subnormal diets. The findings was similar to the report by Nodu *et al*., (2014) who reported significantly higher values of RBC, WBC, Hb and PCV when pawpaw leaf meal was fed to young rabbits. Pawpaw and

its part were reported to be rich in minerals, which are useful in the coagulation of blood, proper functioning of the heart and nervous system, purifies the blood and normal contraction of the muscles (Atta, 1999). A lot of research have reported normal ranges for haematological parameters in avian species. Bounous and Stedman (2000) recorded normal haematological parameters of PCV (30 – 40 %), Hb (7 – 13 g/dl), RBC (3.4 – 4.6 x 10^3 ul) and WBC (12 – 30 x 10^3 ul) in broiler chickens. Furthermore, Banjoko *et al*., (2020) also recorded a normal haematological parameters of PCV (24.90 – 45.20 %), Hb (7.40 – 13.10 g/ dl), RBC (1.58 – 4.10 x 10^{12}/ l) and WBC (9.20 – 31.00 x 10^{12}/ l).

2.14 Serum biochemical Activity of *Carica papaya* (Pawpaw)

The liver is the centre of several digestive, metabolic and productive activities, and as such, is susceptible to varying degree of chemical and biological changes. Such changes or damages are made obvious by the serum levels of specific enzymes originating from the liver. These enzymes depending on their levels may cause some disruptions in bodily functions, thereby resulting in poor health and performance. Enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) in the blood are bio-indicators of liver function and damage (Yildirim *et al*., 2011). *Carica papaya* (pawpaw) is reported to play a role in the serum biochemical parameters of a chicken. Bolu *et al*. (2009) reported significant difference in the values of glucose, uric acid, serum protein, albumin, creatinine, GOT and GPT when varying levels of dried pawpaw seeds at 0, 5, 10 and 15 % were fed to broiler chickens. On the contrary, Henry *et al*. (2017) reported no significant difference on total protein, urea, albumin, globulin when weaned rabbits were fed pawpaw leaves as feed supplement (as fresh and as wilted pawpaw leaves). Similarly, Umar and Muhammed (2020) reported no significant difference in AST, ALT, ALP, Bil-total, Bil-direct albumin, total protein, creatinine, urea, potassium chloride and sodium when broiler chickens were fed graded levels of *C.*

papaya seed powder at 0, 0.50, and 1 %. This variation might be as a result of differences in the methods of processing of pawpaw plant parts as well as doses administered to the birds.

2.15 Immunomodulatory and Histopathology Activity of *Carica papaya* (Pawpaw) Seeds

The immune defense toward infections is essential for maintaining the health of poultry. Traditionally, *C. papaya* seed has been used to improve the immune system of people. In *in vivo* experiment, *C. papaya* seeds extract increased the responsiveness of lymphocytes to phytohemagglutinin and inhibited the classical complement-mediated hemolytic pathway (Mojica-Henshaw *et al*., 2003). In view of this, Amazu *et al*. (2010) documented a substantial inhibition (57.1 - 64.2 %) of *C. papaya* seeds extract on the inflammation in rat model when induced with egg albumin. Furthermore, Oloruntola (2019) reported marked proliferation of polymorphonuclear and mononuclear cells, activation of liver microphages system and the kupffer cells in the liver of broiler chickens when fed pawpaw leaf and seed meal composite mix dietary supplementation. *C. papaya* seeds extract has been revealed at the concentration of 10 mg/ml to inhibit the expression of interferon-gamma, tumor necrosis factor-alpha, interleukin-6, and nuclear factor-κB in pancreatic (HPDE-6) epithelial cells stimulated with methyl isocyanate (Pathak *et al*., 2014). On the contrary, Omidiwura *et al*. (2020) reported eroded villi of mucosa layer when *C. papaya* leaf meal diet at 3 % and combination of 1.5 % *C. papaya* and 1.5 % *Chromolaena odorata* leaf meal diet showed severe infiltration of inflammatory cells of the gut of broiler chicken. However, certain bioactive components have been suggested to play a vital role in immunomodulatory and anti-inflammatory activities of *C. papaya* seed. Kadiri *et al*. (2016) suggested that enzymes, vitamins, polyphenols, and other active components are attributed to the pharmacological and immune-modulatory properties of

C. papaya seeds. In view of this, the enzymes (papain and chymopapain) and flavonoid in *C. papaya* seeds could regulate the inflammatory markers that in turn, modulate the immune responses and defend host against pathogens (Pandey *et al*., 2016). In rabbits, it was also reported that *C. papaya* seeds increased the count of platelets, which are essential components of the immune system (Asadullah *et al*., 2017). Furthermore, *C. papaya* seed also contains minerals (specifically zinc), that may be essential for the immune modulation of host (Moses and Olanrewaju, 2018). Similarly, bioflavonoids in *C. papaya* seeds may strengthen the host immune system. It was reported that some bioactive compounds in *C. papaya* seeds works in synergy to exert immunomodulatory activities in host (Mojica-Henshaw *et al*., 2003).

2.16 Description of Causative Agents of Coccidiosis

Parasites causing coccidiosis are commonly found in places where chickens are raised. The most common means of coccidial parasites spreading is related to the movement of personnel and animals between houses and farms. Coccidiosis is a self-limiting disease, and its manifestation depends on the number of oocysts ingested and the immune status of the host bird (McDougald and Fitz-Coy, 2009). Avian coccidiosis is caused by several *Eimeria species* (family *Eimeriidae*) that belong to the phylum *Apicomplexa*. This phylum comprises many members of entirely parasitic diseases of humans and animals with a wide environmental distribution. Organisms belonging to this phylum are obligate intracellular parasites characterized by unique specialized organelles, notably those from within the apical complex (micronemes, rhoptries, dense granules, and conoid and polar rings), that would provide the structural stability required during the host invasion process (Morrison, 2009). Over 5000 species of *apicomplexan* parasites have been reported, including *Plasmodium falciparum*, the causative agent of malaria; *Babesia* and *Theileria*, cattle parasites; *Cryptosporidium*, *Toxoplasma*, and *Sarcocystis*, human and animal

pathogens; and *Eimeria spp*., poultry and cattle pathogens (Hu *et al*., 2006). The damage caused by these parasites is host-specific and is based on the uncontrolled cycles of host cell invasion, parasite proliferation, host-lysis, and reinvasion. More than 1000 species of *Eimeria* are reported and most parasitize the intestinal epithelia of vertebrates including cattle, sheep, goat, rabbits, horses, domestic dogs and cats, pigs, turkeys, and chickens, with an economically significant burden (Witcombe and Smith, 2014).

In the domestic fowl (*Gallus gallus*), nine different *Eimeria spp*. are documented (Morgan *et al*., 2009 and Haug *et al*., 2008). *Eimeria spp.* invade and destroy the intestinal epithelium of poultry birds, and as a result, infected birds display reduced feed intake, bloody diarrhea, and reduced weight gains (Gilbert *et al*., 2011). *Eimeria* parasites develop in different regions of the gut and depending on the magnitude of the infection; they can cause mild to severe lesions. Each species of *Eimeria* parasite is specific to a particular site in the gastrointestinal tract. For instance, *E. acervulina* develops in the duodenum, *E. maxima* and *E. mitis* develop in the middle part of the small intestine, *E. tenella* develops in the caeca, *E. brunetti* develops in the caeca and the rectum, and *E. necatrix* develops in the small intestine (Raman *et al*., 2011). The species of *Eimeria* that are reported as highly pathogenic are *E. brunetti*, *E. maxima*, *E. necatrix,* and *E. tenella*. Species reported as mildly pathogenic include *E. acervulina*, *E. mitis*, and *E. mivati*, while *E. praecox* and *E. hagani* are considered to be the least pathogenic (Jadhav *et al*., 2011).

2.17 The Life Cycle of *Eimeria species*

The life cycles of *Eimeria species* are complex, consisting of two developmental stages in the host: an exogenous stage (*sporogony*) and an endogenous stage (*schizogony* and *gametogony*). Some species vary in the number of asexual generations and the time required for each developmental stage (Blake and Tomley, 2014). During the exogenous phase, the unsporulated (non-infective) oocyst is excreted from the chicken and

undergoes sporulation in the presence of moisture, warmth, and oxygen, thus becoming a sporulated (infective) oocyst. Sporulated oocysts of *Eimeria* contain four sporocysts, each containing two sporozoites. The endogenous phase occurs in the intestine of the host and involves several rounds of asexual reproduction (*schizogony*) followed by sexual differentiation (*gametogony*), fertilization, and the shedding of an unsporulated oocysts (Lal *et al*., 2009). The last two generations of asexual development (sometimes as many as four) give rise to a sexual phase, where small, motile microgametes seek out macrogametes to form the zygote which matures into an oocyst that is released from the intestinal mucosa and shed in the faeces. Though, the reproductive potential of a single ingested oocyst is fairly constant (McDougald, 2013). The short life cycle (4 - 6 days, depending on the species) and large production of sporulating oocysts are advantageous for increasing the chances of infecting a large population of chickens. Infection occurs when the host ingests sporulated oocysts. Following ingestion, the microenvironment of the host digestive tract stimulates transformation of the oocyst in the gizzard resulting in the release of sporozoites that invade and destroy cells in the intestinal mucosa and begin the reproductive cell cycle. As a result, infected birds display symptoms of disease such as reduced feed intake, bloody diarrhoea, and reduced weight gain (Gilbert *et al*., 2011).

2.18 Gross Pathology of Birds Infected with *Eimeria species*

Birds infected exhibit lethargy, diarrhoea, drooping wings, muscle wastage and sudden death. Previous exposure to infection and the number of oocysts ingested all contribute to disease severity. Pathology is caused by the acute destruction of the intestinal mucosa during invasion and departure of the sporozoites and the merozoites respectively. *E. necatrix* and *E. tenella* are the most pathogenic parasites because of the location of schizogony (the asexual reproduction into merozoites), which occurs in the small intestine and the ceca, causing extensive haemorrhage (James *et al*., 2009).

2.19 Mechanism of Cell Invasion by *Eimeria species*

Although the invasion of the intestinal cell seems important to the pathology, and a potentially useful point for intervention, the mechanisms involved are not well determined. The invasion of the protozoa into the intestinal epithelium occurs in the complex environment of the intestinal lumen, because of this, it is difficult to isolate the processes which are involved exclusively in the entry of the protozoa into the host cell. It is reported that susceptible target cell recognition and cell invasion is the first step in establishing an active infection and disease in the host animal (Augustine, 2001). It is also known that the host cell recognises certain surface antigens (specific proteins) on the sporozoites which induces a process of internalisation by the host cell (Augustine, 2001). There have been a number of theories proposed for how this takes place but all agree that secretions produced by the anterior end and organelles of all *Apicomplexa* are essential for the recognition, attachment and invasion of the host cell (Augustine, 2001). Invasion is initiated by contact between the cell surface and the anterior end of the sporozoites; then by a process of membrane internalisation the parasite is enclosed by a parasitophorous vacuolar membrane (PVM) (Min *et al*., 2004). *Eimeria spp.* contains several apical organelles including micronemes (which secrete soluble antigens which are released during host cell invasion), dense granules and rhoptries (Dubremetz *et al*., 1998). It is believed that these secreted proteins coat the parasite and form a connection between the parasites actinmyosin cytoskeleton and the host cell surface, aiding invasion (Bumstead and Tomley, 2000). After *Eimeria* sporozoites invade the host cell they inhabit a parasitophorous vacuolar membrane (PVM), derived from the plasma-lamella of the host cell (Beyer *et al*., 2002).

2.20 Genotoxicity and Cytotoxicity of *Eimeria species*

A number of parasites excrete substances which have been revealed to induce cytotoxic and genotoxic damage to cells. *E. tenella* has been reported to weaken antioxidant status during the course of infection and causes oxidative stress after infection (Georgieva *et al*., 2006). The ability for protozoa to cause DNA damage to target cells has been demonstrated for *Plasmodium falciparum* and *Schistosoma haematobium*. Nonetheless, research relating to the ability of coccidial parasites to cause DNA damage has been limited to one study looking at *Toxoplasma* genotoxicity on mouse brain, liver and peripheral blood cells (Ribeiro *et al*., 2004). It was also discovered that *T. gondii* induced DNA damage in peripheral blood cells. However, no studies have been reported for genotoxicity of *Eimeria*, or explored in any detail what cytotoxic factors may be involved in cellular invasion (James *et al*., 2009).

2.21 Detection of Cellular Damage by *Eimeria species*

No doubt some parasites may have genotoxic effects, a reliable method for detecting DNA damage caused by parasites such as *Eimeria* in individual cells is essential. One potential technique for this task is the single gel electrophoresis (also called comet assay). The technique allows sensitive detection of DNA damage to individual cells. It can also be used for detecting single or double-stranded breaks in DNA. The technique has become popular for genotoxicity testing in a variety of situations including environmental contamination (Knopper and McNamee, 2008), toxological studies and radiation exposure (Hasan *et al*., 2008). The technique is also useful for monitor-ring DNA damage over a period of time. This report is particularly focused on the application of the comet assay to the cellular damage caused by the coccidial parasites. The technique involves electrophoresis of cells embedded in an agarose gel. After chemical lysis of the treated cells with a strong salt and detergent solution, the electrical current causes broken DNA

strands to migrate. The spread of different DNA strands forms the shape of a comet (from which the assay takes its name). Under the microscope, using a suitable nuclear stain, intact DNA will be visible as the 'head' of the comet and damaged DNA which will migrate to form the 'tail'. The amount of DNA in the 'tail' and hence the amount of damage present can be calculated using a wide range of softwares. The method can be conducted under neutral conditions which detects single and double strand breaks, or more commonly under alkaline conditions which detect both of the above but will also resolve the cross-links, the base damage and the apoptotic nuclei. Despite the popularity in the field of genotoxicity, the method has not yet been widely applied to the study of parasitic damage (James *et al*., 2009).

2.22 Management, Nutrition and Diets of Broiler Chickens

Broiler chickens are mainly reared for fast growth and development and slaughtered when they weight about 1.8 to 2.2 kg live mass, usually between 6 and 8 weeks of age (Musa *et al*., 2006). In general, the main objective for broiler chicken producers is to produce meat with acceptable lipid content and learner tissues in order to meet modern consumer demands (Weltzien, 2009). Although, feed expenditures frequently comprise 80 % of broiler production cost, decisions concerning ration composition have a significant impact upon profitability of any broiler production enterprise. However, ration composition is one among many interactive components that must be met for efficient production (Louw *et al*., 2011). Amakari and Owen (2011) reported that to ensure fast growth rate and efficient feed conversion in broiler production, effective disease prevention and control including good management practices, provision of high quality feed and clean and fresh water (*ad libitum*) as well as flock maintenance under continuous lighting are all vital.

2.23 Blood

Blood is a specialized body fluid in animals and humans that transports vital substances such as oxygen and nutrients to the cell and remove metabolic waste from the cell. In vertebrate, blood is composed of blood cells suspended in liquid called plasma. The plasma constitutes 55 % of blood fluids which is mainly water (92 % by volume) and it contain dissolved protein, hormones, mineral ions, glucose, oxygen, carbon dioxide and blood. The blood cells constitute primarily the red blood cell (erythrocytes), white blood cell (leukocytes) and platelets (thrombocytes). The red blood cells are the most abundant cells in the vertebrate. The blood is circulated around the body through blood vessels by the pumping action of the heart cell (Kral and Suchy, 2000).

Blood examination is used to determine physiological and biochemical status such as mineral content, health status, drug effectiveness and organ function. Blood examination also provides substantial values in poultry rearing enterprises. For instance, provision of information on the assessment of poultry's health such as injury, diseases, parasitism, bacteria septicemia, nutritional deficiency and physiological changes in growth and development (Jain, 1993). Haematological evaluation is vital for provision of information on immune status, diagnostic and management purposes. It can be used as diagnostic tools in order to determine the health status of an individual or flock. Haematological changes are usually used to determine the body status and to assess the impact of the environment, nutrition and pathological stress. Biochemical profile is a blood test that assesses the function of the internal organs, measures the electrolytes such as blood potassium and identifies the levels of circulating enzymes (Kral and Suchy, 2000).

2.23.1 Hematological indices

2.23.1.1 *Red blood cell (erythrocytes)*

The red blood cell (erythrocytes) contains haemoglobin that carries oxygen. The oxygen in the tissue depends upon the quantity of red blood cells and how well they function. The red blood count is part of the complete blood test (CBC). This test can help diagnose anaemia. Higher than normal number of red blood cells may be due to; dehydration, kidney tumor, low blood oxygen levels, nutritional deficiency of iron, copper, foliate, vitamin B6, vitamin B12 and excess hydration (Uthman, 2007).

2.23.1.2 *Packed cell volume (PCV)*

The packed cell volume is the volume or percentage (%) of the red blood cells in the blood. It is considered as the fundamental part of the complete blood count results. In mammals, erythrocytes volume fraction is dependent on the body size. Increased packed cell volume leads to polycythemia vera (PV), a myelo-prolifera disorder in which the bone marrow produces excessive number of red cells, and chronic obstructive pulmonary diseases and other pulmonary condition associated with hypoxia which may elicit an increased production of red blood cells. Lowered packed cell volume can result to haemorrhage (Purves *et al*., 2004).

2.23.1.3 *White blood cell (leukocytes)*

White blood cells are cells of the immune system which involve defending the body against both infectious diseases and external materials. They are produced in the bone marrow. Decreased numbers of white blood cells are called leucopenia, which may be due to the bone marrow failure or deficiency, collagen-vascular diseases, diseases of the liver or spleen. Elevated numbers of white blood cells are called leukocytosis. It may be

as a result of infectious diseases, anaemia, bone marrow tumour and tissue damage (Bagby, 2007).

2.23.1.4 *Haemoglobin*

Haemoglobin is an iron-containing compound found in the red blood cells which transport oxygen around the body. Haemoglobin in the blood carries oxygen from the lungs to the rest of the body. It has an energy binding capacity of 1.34 ml oxygen per gramme of haemoglobin. Measuring the concentration of haemoglobin in blood can help diagnose anaemia (Uthman, 2007).

2.23.1.5 *Lymphocytes*

Lymphocytes are usually found in the lymphatic system and are distinguished by having a deeply staining nucleus that may be eccentric in the location and relatively small amount of cytoplasm. Blood have three (3) types of lymphocytes

i. **T cells** – These cells are responsible for the regulation of the antigens and cell mediated response of the animal.
ii. **B cells** – These cells provide antibodies that bind to pathogens to enable their destruction.
iii. **Natural killer cells** – These cells are able to kill cells of the body that have lost MCHC molecules as they have been infected by virus or have become cancerous. Lymphocytosis is generally an increase in the number of lymphocyte, known as lymphocytopenia (Abbas and Linchtman, 2003).

2.23.1.6 *Mean corpuscular haemoglobin concentration (MCHC)*

This can also be called mean corpuscular haemoglobin. It is a measure of the concentration of haemoglobin in a given volume of packed red blood cells. It further guides to the investigation of anaemia. It was reported also as part of standard complete

blood count. MCHC is calculated by dividing the haemoglobin by the haematocrite. Mean corpuscular haemoglobin concentration can be low when there is iron deficiency, blood loss and anaemia caused by chronic diseases (VanBeckvelt *et al*., 2001).

2.23.1.7 *Mean corpuscular volume (MCV)*

Mean corpuscular volume is an estimate of the volume of the red blood cells. It is useful for determining the types of anaemia in animals. Low mean corpuscular volume may signify chronic diseases, iron deficiency haemoglobin disorder. Increased mean corpuscular volume may indicate anaemia due to bone marrow abnormalities, chronic lung diseases and nutritional deficiencies. (Jaime and Howlet, 2008).

2.23.1.8 *Neutrophils*

Neutrophils functions to defend the body against bacterial infections and other very small inflammatory processes that are commonly first responders to microbial infection. Their activities and death in large numbers form pus. Neutrophils are active in phagocytosing bacteria and are present in large amounts in the pus of wound (Jaime and Howlet, 2008).

2.23.1.9 *Monocytes*

Monocytes share vaccum cleaner (phagocytosis) function of neutrophils and are longer lived as they have an additional role. They present pieces of pathogens to T-cells so that the pathogen may be recognized again and killed. Monocytes are produced by the bone marrow and constitute 3 – 8 % of leukocytes in the blood (Jaime and Howlet, 2008).

2.23.1.10 *Basophils*

Basophils plays a main role in both allergies and parasitic infections. They are responsible for antigens and allergic response by releasing the chemical histamine causing vasodilation. The nucleus is bi- or tri- lobed and are hard to see it because of the number

of coarse granules. Basophils also contain anticoagulant heparin responsible for preventing blood from clothing too fast (Jaime and Howlet, 2008).

2.23.1.11 *Eosinophils*

Eosinophils majorly deal with parasitic infections. They predominate inflammatory cells in allergic reactions. The causes of eosinophilia include allergies such as asthma, hay fever and hive and parasitic infection. Eosinophil consists of 1.6 % of white blood cells. In general, their nucleus is bi-lobed and the cytoplasm is full of granules that assume a characteristic pink-orange colour with eosin stain (Young *et al*., 2006).

2.23.2 Serum biochemistry parameters

2.23.2.1 *Albumin*

Albumin is the most important protein found in the body. It conveys various substances through the blood and it is essential in maintaining pressure within the vessels. Elevated levels indicate dehydaration. Low level indicates liver diseases, kidney diseases, inflammation, starvation and blood loss (Wang *et al.*, 2012).

2.23.2.2 *Total protein*

Total protein is an important substance in all part of the body. High level indicates dehydration, chronic infection, inflammation and certain cancer. Low level indicates liver diseases, intestinal absorption problem and losses via the kidney (Wang *et al*., 2012).

2.23.2.3 *Aspartate amino transferase (AST)*

Aspartate amino transferase (AST) is commonly found among tissues such as heart, kidney, liver, muscles and brain. It is discharged into the serum when any of the tissue is damaged. Elevated level in the blood may indicate liver damage. It may also indicate gall bladder diseases, infection or viral hepatitis (Wang *et al*., 2012).

2.23.2.4 *Alanine amino transferase (ALT)*

This enzyme is found in serum and in various tissues but usually associated with the liver. It is measured clinically as part of diagnostic evaluation of hepatocellular injury to determine liver health (Wang *et al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experiment One: *In vitro* treatment of coccidial organisms in broiler chickens using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

3.1.1 Study site

The *in vitro* experiment was conducted in Animal Production Laboratory of the School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan Kwano, Minna, Niger State, Nigeria. Minna is located between latitude $90^{0}37^{/}$, North and longitude $6^{0}33^{/}$, East of the equator. Minna has a mean annual rainfall of 1,200 mm, with the average highest temperature in the month of March and lowest temperature in the month of August. The mean annual temperature is between 22^{0}C to 40^{0}C. Minna is located in the Southern Guinea Savanna vegetation belt of Nigeria and has two distinct seasons; wet season from March to October and dry season from November to March (Ovimap, 2015).

3.1.2 Preparation of ripe pawpaw seed extracts

Fresh ripe pawpaw (*Carica papaya*) seeds of mixed varieties were collected, washed thoroughly, air dried for four (4) weeks and ground to fine powder with an electric grinding machine and stored in a polythene bag until ready for use. Five kilogram (5 kg) ground-fine powdered seeds of ripe pawpaw (*Carica papaya*) was used for the experiments (experiment 1 and 2). One hundred grams (100 g) of stored powder was macerated in 1 litres of ethanol and stirred at 3 hours interval for 72 hours, and then filtered using Whatman Paper No. 3. The filtrate was concentrated by evaporating the solvent at 75^{0}C using a rotatory evaporator (Buchi R-200) to obtain the extract and kept in a refrigerator at 4^{0}C until ready for use.

3.1.3 Procedure of phytochemical analysis

Phytochemical analysis of ethanolic extract of ripe pawpaw seeds (EERPS) was carried out to determine the presence of phenol (Keay *et al*., 1964), flavonoids, saponin, alkaloids and tannin (Innocent *et al*., 2021). The following are the quantitative procedures of phytochemical analysis:

3.1.3.1 *Determination of phenol*

Defatting of 2 g of the sample (ripe pawpaw seed powder) was carried out for 2 hours in 100 cm^3 of ether using a soxhlet apparatus. The defatted sample (0.5 g) was boiled for 15 minutes with 50 cm^3 of ether for the extraction of the phenolic components. Precisely 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide solution, 5cm^3 of the concentrated amyl alcohol was added to 5 cm^3 of the extract and allowed to react for 30 minutes for colour development. The optical density was measured at 505nm. 0.2 g tannic acid was dissolved in distilled water and then diluted to 200 ml mark (1 mg/ cm^3) in preparation for phenol standard curve. Varying concentration (0.2 - 1.0 mg/ cm^3) of the standard tannic acid solution were pipette into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water were added. The solution was made up to 100 cm^3 volume and allowed to react for 30 minutes for colour development. The optical density was determined at 505 nm (Keay *et al*., 1964).

3.1.3.2 *Determination of flavonoids*

A known quantity of 1.0 g of the sample was repeatedly extracted with 100 ml of 80 % ethanol at room temperature. The solution was shaken for 30 minutes and filtered. The filtrate was transferred into a weighed beaker and evaporated to dryness over a water bath and weighed again. The time for the first extraction was 1 hour, 45 minutes for the second

extraction and 30 minutes for the third extraction. Flavonoid was determined using the following formula;

$$\text{Concentration of flavonoid} = \frac{W_2 - W_1}{W_3} \qquad \text{Eq. 3.1}$$

Where, W_1 = Weight of empty beaker

W_2 = Weight of beaker + Sample after drying,

W_3 = Weight of sample used

3.1.3.3 *Determination of saponins*

A known quantity of 1.0 g of the sample (ripe pawpaw seed powder) was weighed using electric weighing balance into a 250 ml beaker and socked with 100 ml of 20 % ethanol for 3 minutes and heated for 3 hours at 55^0C for proper extraction, then filtered. The residue was re-extracted with another 100 ml of 20 % ethanol. The two extracts were combined and heated to 40 ml at 90^0C on a water bath. The concentration was transferred into 500 ml separating funnel and 200 ml of diethyl ether was added and properly shaken, and the upper layer was discarded. The purification process was repeated, and 60 ml of n-butanol was added. The lower layer was discarded while the upper layer was collected. The combined n-butanol extract was washed with 10 ml of 5 % aqueous NaCl and the lower layer discarded while the layer was collected in the weighed beaker and heated to dryness. The beaker was allowed to cool in a desiccator and reweighed. The saponin content was determined using the following formula:

$$\text{Concentration of saponin} = \frac{W_2 - W_1}{W_3} \qquad \text{Eq. 3.2}$$

Where, W_1 = Weight of empty beaker

W_2 = Weight of beaker + Sample heating

W_3 = Weight of sample used

3.1.3.4 *Determination* of alkaloids

A known quantity of 1.0 g of the sample (ripe pawpaw seed powder) was weighed using the electric weighing balance into 250 ml beaker and 100 ml of 10 % acetic acid into ethanol. The mixture was allowed to stay for 4 hours for proper extraction. The sample was filtered with filter paper and the extract was concentrated in a water bath to one quarter of the original volume. A volume, 20 ml of ammonium hydroxide (NH_4OH) was drop wisely added to form precipitate of the alkaloid in the filtrate. The filtrate was weight with the NH_4OH and filtered. The filter paper and the precipitate were dried in an oven at 40^0C and weighed. The alkaloid content was determined using the following formula:

$$\text{Concentration of Alkaloid} = \frac{W_2 - W_1}{W_3} \qquad \text{Eq. 3.3}$$

Where, W_1 = Weight of empty filter paper

W_2 = Weight of the Alkaloid and filter paper

W_3 = Weight of the sample used

3.1.3.5 *Determination of tannins*

A quantity of 1.0 g of the sample (ripe pawpaw seed powder) was weighed into a plastic container and 50 ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was then filtered into 50 ml volumetric flask to a mark. A volume, 5 ml of the filtrate was dispensed into a test tube mixed with 2 ml of 0.1 M $FeCl_2$ in 0.1 NHCl and 0.008 M Potassium ferrocyanide, the absorbance was measured at 720 nm for 10 minutes. The tannin concentration was determined using the following formula:

Concentration of tannin = $\frac{\text{Abs x DF}}{\text{1000 x Weight of sample used}}$

Where, Abs = Value of absorbance read

DF = Dilution factor

3.1.4 Preparation of culture media (potassium dichromate $K_2 Cr_2 O_7$)

A quantity of 2.5 % potassium dichromate was prepared by dissolving 2.5 g of potassium dichromate in 100 ml of distilled water. This culture medium was stored and used to prepare extract concentrations (Yamssi *et al*., 2017).

3.1.5 Preparation of Hanks buffered salt solution (HBSS)

Buffer HBSS:

KCl ……. 0.4 g

KH_2PO_4 …….. 0.06 g

NaCl ……. 8.0 g

$NaHCO_3$ ……..…… 0.35 g

Na_2HPO_4 …… 0.048 g

D-glucose ……... 1.0 g

Water was added up to 1litre and the buffer frozen for storage (Yamssi *et al*., 2017).

3.1.6 Isolation of Eimeria oocysts

The oocysts were isolated from infected faecal samples of infected birds obtained from farms within Minna metropolis. The faecal samples were collected, stored in an air-dried polythene bag and transported to Animal Production Laboratory of the School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan

Kwano, Minna for examination. Ten grammes (10 g) of the faecal sample was dissolved in 20 ml of 2.5 % potassium permanganate and mixed homogenously and allowed to stay for 30 minutes. The mixture was filtered using muslin cloth and the filtrate collected. The filtrate was added with saturated sodium chloride (NaCl) to bring about precipitation of the oocysts. The mixture was centrifuged at 350 rpm for 15 minutes. The suspended oocysts were collected using cannula. The oocysts were suspended in the HBSS buffer until ready for use (Heelan and Ingersoll, 2002).

3.1.7 *In vitro* treatment of extract on isolated coccidial organisms

The extract of ripe *Carica papaya* (pawpaw) seeds was prepared at different concentrations of 20, 30, 40 and 50 mg/ ml and dissolved with Dimethyl sulphoxide (Dms). The diluted extract was transferred into petri dishes and equal volume of oocysts (2 ml) was placed in each and then incubated at 28 - 30^0C for 24 - 48 hours. The sporozoites were counted using malassez counting chamber. The number of viable sporozoites and non-viable sporozoites were estimated by counting the number of sporozoites in a total of 100 oocysts. The sporozoites percentage was calculated as follows:

$$\text{Percentage (\%) Sporozoites } \frac{\text{\% Viable Sporozoites} - \text{\% Non} - \text{Viable Sporozoites}}{\text{\% Viable Sporozoites}} \text{ X } 100$$

(Yamssi *et al*., 2017) Eq. 3.4

3.2 Experiment Two: *In vivo* treatment of coccidial organisms in broiler chickens using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

3.2.1 Study site

The *in vivo* experiment was conducted in the Poultry Production Unit, Ministry of Livestock and Fisheries Bosso, Minna, Niger State, Nigeria. Minna is located between latitude $90^{0}37^{/}$, North and longitude $6^{0}33^{/}$, East of the equator. Minna has a mean annual rainfall of 1,200 mm, with the average highest temperature in the month of March and lowest temperature in the month of August. The mean annual temperature is between 22^{0}C to 40^{0}C. Minna is located in the Southern Guinea Savanna vegetation belt of Nigeria and has two distinct seasons; wet season from March to October and dry season from November to March (Ovimap, 2015).

3.2.2 Source of experimental materials

One Hundred and fifty (150) day-old chicks were obtained from a reputable hatchery (CHI), Ajanla Farms Limited, Ibadan, Oyo state. Fresh ripe pawpaw (*Carica papaya*) seeds were obtained from the fruit section of Kure-ultra modern market Minna, Niger State. Commercial feed (ultima poultry feed) was purchased from Auwal Ibrahim Road, Mypa junction Bosso, Minna, Niger state.

3.2.3 Experimental design and treatments

The experimental birds were randomly allocated into five (5) treatments, each treatment consisting of three (3) replicates with ten (10) birds per replicate in a completely randomized design (CRD). All treatments were subjected to the same environmental conditions with feed and water provided *ad libitum*. The experiment included the starter phase (1-4 weeks) and finisher phase (5-8 weeks). At week four (4) to week eight (8), varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds at 2, 3, 4, and

5 g/ litre of drinking water, were administered orally at weekly interval to experimental birds in T2, T3, T4 and T5 respectively. However, T1 received amprolium 250 which served as control and was administered at 1 g/litre of drinking water.

3.2.4 Management of experimental birds

Day old experimental birds were reared under uniform brooding condition on arrival from the hatchery. An open sided wall house was used and experimental birds were managed on deep litter system. Prior to arrival of the birds, the pen was subjected to thorough cleaning and removal of debris and cobwebs, followed by scrubbing and washing with disinfectant (Izal solution). The building was demarcated into 15 compartments with woods and net. Wood shavings were spread on the floor at a depth of 5cm and equipments such as pilot light, drinkers, feeders and heating device such as charcoal pot were provided. Routine management such as washing of feeders, drinkers, observation of the chicks for any chick that need attention such as weakness, disease infection, cleaning of litter and other chores were adhered to as recommended by Oluyemi and Robert (2000). Broad Spectrum antibiotic was used as recommended by the manufacturer, for disease treatment and vaccination such as Gomboro, 7 days; Lasota, 14 days; Gomboro booster, 21 days and Lasota, 28 days; were also administered.

3.2.5 Determination of proximate composition of ripe *Carica papaya* seed extract and commercial feeds

Crude protein, ether extract, moisture content, ash content, crude fibre and nitrogen free extract contained in ripe *C. papaya* seed extract and commercial feed were determined by the method described by Onwuka (2005).

3.2.6 Data collection

3.2.6.1 *Performance evaluation of experimental birds*

The following parameters of the broiler chicken birds were measured and recorded: initial body weight of the birds, weekly body weight gain, daily feed intake, feed conversion ratio and mortality rate.

3.2.6.2 *Initial body weight*

The experimental birds were weighed with salter digital weighing scale (SF 400) at week three (3) and result was recorded. The birds were weighed collectively in a replicate and dividing the weight obtained by the number of the birds in each replicate.

$$\text{Initial body weight} = \frac{\text{Total weight of birds (replicate)}}{\text{Number of birds (replicate)}} \quad \text{Eq. 3.5}$$

3.2.6.3 *Weekly body weight gain*

This was determined as the difference between the previous weight gain and the present weight gain after a week. The weekly body weight gain was divided by the number of birds in a replicate to obtain average weekly body weight gain per bird per replicate.

Weekly body weight gain = present week body weight (g) – previous week body weight (g) (Muhammed *et al*., 2012).

$$\text{Average body weight gain/bird/replicate/week} = \frac{\text{Weekly body weight gain (g)}}{\text{Number of birds/replicate (g)}} \quad \text{Eq. 3.6}$$

3.2.6.4 *Daily feed intake*

Daily Feed intake per day per replicate treatment group (pen) was estimated as the difference between quantity of feed offered and quantity of left over feed after 24 hours.

Total feed intake = Total feed offered (g) – Total feed left over (g)

3.2.6.5 *Feed conversion ratio (FCR)*

Feed conversion ratio on the experimental birds was calculated as feed consumed divided by weight gain for each replicate using the following expression:

$$\text{Feed conversion ratio} = \frac{\text{Average weekly feed intake(g)}}{\text{Average Weight gain/ week(g)}} \quad \text{Eq. 3.7}$$

3.2.6.6 *Percentage (%) mortality*

Percentage (%) mortality on the experimental birds was calculated by taking the ratio of number of dead birds to total number of stocked birds per replicate, multiplied by 100, as follows

$$\text{Percentage (\%) mortality} = \frac{\text{Number of dead birds}}{\text{Total Number of stocked birds}} \times 100 \quad \text{Eq. 3.8}$$

3.2.7 Examination of faecal samples

At weeks 3, 5 and 8 of the experiment, faecal samples from the experimental birds were collected for examination of the level of *Eimeria* species, by floatation method using saturated solution of sugar as described by Sharma *et al*. (2013).

3.2.8 Blood collection

At week 3, 5 and 8, two (2) birds were selected randomly from every replicate for blood sample collection using syringe and needle. 3ml of blood was collected from the wing vein of the birds. 1ml of blood was collected into heparinized tubes containing anticoagulate (Ethylene diamine tetra-acetate, EDTA) for haematological indices (such as red blood cell count, white blood cell count, haemoglobin concentration and packed cell volume) while 2 mls were collected into plain vials for liver enzymes assay (alanine

amino transferase, aspartate amino transferase and alkaline phosphatase) and biochemical profile (serum creatinine, total protein and serum albumin).

3.2.8.1 *Haematological indices*

The red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb) and packed cell volume (PCV) were determined as described by Ewuola and Egbunike (2008).

3.2.8.2 *Serum biochemical profile*

Parameters including alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), serum creatinine, total protein and serum albumin were determined using the methods described by Bahman *et al.* (2011).

3.2.9 Histopathology of the gastrointestinal tract

Sections of the gastrointestinal tract (GIT), particularly the caecum from birds on each of the treatment groups (1bird/ replicate), were taken for histological examination of possible lesions according to the procedure of Auwioro (2010). The biopsis of the GIT were fixed with 10 % formal saline, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with haematoxylin and eosin staining technique and examined with Leica ICC 50HD microscope and photographed with Leica ICC 50HD camera. The photomicrographs were transferred to the computer for further readings.

3.2.10 Data analysis

Data obtained were subjected to analysis of variance (ANOVA) using the general linear model procedure of SPSS (2021) version 23. Treatment means with significant difference at $P<0.05$ was compared using Duncan Multiple Range Test (DMRT) of the same package.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate composition of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The proximate composition results of ethanolic extract of ripe pawpaw seed (Table 4.1) showed that the moisture content, ash content, crude protein, ether extract, nitrogen free extract values were 30.67 %, 5.33 %, 1.56 %, 0.73 % and 61.71 %, respectively.

4.1.2 Phytochemical composition of ethanolic extract of ripe Pawpaw (*Carica papaya*) seeds

The phytochemical composition of ethanolic extract of ripe pawpaw seed is presented in Table 4.2. The phenol, flavonoids, saponins, alkaloids and tannins were 162.22, 35.18, 99.70, 22.71 and 65.21 mg/ 100 g, respectively.

4.1.3 Proximate composition of the experimental diets

The proximate composition of the experimental diets is presented in Table 4.3. The results showed that the moisture content values of the diets were 8.90 % and 7.48 %, ash content values of 6.14 % and 5.38 %, crude fibre content values of 4.72 % and 5.33 %, crude protein for 23.61 % and 20.48 %, ether extract of 7.21 % and 8.33 %, nitrogen free extract of 49.42 % and 53.00 % and metabolizable energy of 3168.90kcal/ kg and 3279.85kcal/ kg for starter and finisher diets, respectively.

Table 4.1:Proximate Composition of Ethanolic Extract of Ripe Pawpaw (*Carica papaya*) Seeds

Parameters	Quantity (%)
Moisture content	30.67
Ash	5.33
Crude protein	1.56
Ether extract	0.73
Nitrogen free extract	61.71

Table 4.2: Phytochemical Composition of Ethanolic Extract of Ripe Pawpaw (*Carica papaya*) Seeds

Parameters	Quantity (mg/ 100 g)
Phenols	162.22
Flavonoids	35.18
Saponins	99.70
Alkaloids	22.71
Tannins	65.21

Table 4.3: Proximate Composition of the Experimental Diets

Parameters	Starter (%)	Finisher (%)
Moisture content	8.90	4.48
Ash content	6.14	5.38
Crude fibre	4.72	5.33
Crude protein	23.61	20.48
Ether extract	7.21	8.33
Nitrogen free extract	49.42	53.00
Metabolizable Energy (kcal/kg)	3168.90	3279.85

4.1.4 *In vitro* treatment of coccidial organisms using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The effect of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds (EERPS) on *in vitro* treatment of coccidia organisms results is presented in Table 4.4. It showed that the sporozoites recovery rates varies at different concentrations of ripe pawpaw seed extract. Higher sporozoites recovery rate was obtained in control (57.1 %), 20 mg/ml (52.9 %) and 30 mg/ml (50.7 %), respectively. However, the lowest recovery rate was observed at 50 mg/ml with the sporozoites recovery rate of 24.6 %. Though, the results showed significant difference ($P<0.05$) in all the parameters.

Table 4.4: *In vitro* Treatment of Coccidial Organisms Using Ethanolic Extract of Ripe Pawpaw (*Carica papaya*) Seeds

Parameters	T1	T2	T3	T4	T5	SEM	P-value
% Nonviable sporozoites	30.00^{e}	32.00^{d}	33.00^{c}	40.00^{b}	43.00^{a}	1.34	0.00
% Viable sporozoites	70.00^{e}	68.00^{d}	67.00^{c}	60.00^{b}	57.00^{a}	1.34	0.00
% Sporozoites	57.10^{e}	52.90^{d}	50.70^{c}	33.30^{b}	24.60^{a}	3.35	0.00

^{abcde} = Means on the same row having different superscripts are significantly different ($P<0.05$)
T = Treatment, % = Percentage, SEM = Standard error of mean
T1 = 100 % of oocyts in 2 ml treated with distill water
T2 = 100 % of oocyts in 2 ml treated with 20 mg/ ml of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds
T3 = 100 % of oocyts in 2 ml treated with 30 mg/ ml of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds
T4 = 100 % of oocyts in 2 ml treated with 40 mg/ ml of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds
T5 = 100 % of oocyts in 2 ml treated with 50 mg/ ml of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

4.1.5 Growth performance of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The results of the effect of administering varying levels of ethanolic extract of ripe pawpaw seeds (EERPS) on the growth performance of broiler chickens are presented in Table 4.5. The results showed that administering varying levels of EERPS on broiler chickens had no effect ($P>0.05$) on all the growth parameters measured except the final body weight. Broiler chickens in T1 (control), T3 (3g), T4 (4g) and T5 (5g) had similar final body weight values except birds in T2. However, chickens on T1 (control) and T5 (5g) had higher ($P<0.05$) final body weight and body weight gain values than those birds in T2 (2g), T3 (3g), and T4 (4g).

The results of administering varying levels of ethanolic extract of ripe pawpaw seeds (EERPS) on the mortality rate and examination of coccidia oocysts in faecal samples of broiler chickens are presented in Table 4.5. The results showed that administering varying levels of EERPS to broiler chickens had significant effect ($P<0.05$) on the mortality rate and oocysts values. Birds in all treatment groups [T1(control), T2 (2g), T3 (3g), T4 (4g) and T5 (5g)] had similar ($P>0.05$) mortality values and oocysts count values. However, birds in T1 (control) and T5 (5g) had lower ($P<0.05$) mortality values compared to birds in T2 (2g), T3 (3g) and T4 (4g). Similarly, T1 (control) and T5 (5g) had the lowest ($P<0.05$) oocysts count values compared to birds in T2 (2g), T3 (3g) and T4 (4g).

Table 4.5: Growth Performance of Broiler Chickens Administered Varying Levels of Ethanolic Extract of Ripe Pawpaw (*Carica papaya*) seeds

Parameters	T1	T2	T3	T4	T5	SEM	P-V	LS
IBW (g)	810	801	810	880	920	0.15	0.05	NS
FBW (g)	2402^{ab}	2260^{c}	2370^{b}	2370^{b}	2420^{a}	0.15	0.00	*
WBWG (g)	0.33	0.29	0.31	0.30	0.30	0.12	0.86	NS
DFI(g)	10.11	10.10	10.13	10.14	10.15	0.279	1.00	NS
FCR	3.20	5.08	5.12	3.47	3.55	0.43	0.44	NS
Mortality(%)	6.67^{a}	20.00^{c}	16.67^{ab}	13.33^{ab}	10.00^{ab}	1.86	0.04	*
IOC (%)	42.67	41.00	41.67	42.00	42.89	0.540	0.94	NS
FOC (%)	10.67^{a}	20.67^{b}	18.17^{ab}	15.83^{ab}	14.17^{ab}	1.30	0.04	*

^{abc} = Means on the same row having different superscripts are significantly different ($P<0.05$), * = Significant, NS = Not significant, SEM = Standard error of mean, LS = Level of Significance, T = Treatment, T1 = Amprolium, T2 = 2 g/ litre, T3 = 3 g/ litre, T4 = 4 g/ litre, T5 = 5 g/ litre, IBW = Initial body weight, FBW = Final body weight, WBWG = Weekly body weight gain, DFI = Daily feed intake, FCR = Feed conversion ratio, IOC = Initial oocysts count, FOC = Final oocysts count, P V= Probability value

4.1.6 Haematological parameters of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The results of the effect of administering varying levels of ethanolic extract of ripe pawpaw seeds (EERPS) on the hematological parameters of broiler chickens are presented in Table 4.6. The results showed that administration of varying levels of EERPS to broiler chickens had significant effect ($P<0.05$) on RBC values while WBC, PCV and HC were not influenced ($P>0.05$) upon EERPS administered to the birds. RBC values of T1, T4 and T5 were similar but significantly higher than values for T2 and T3.

4.1.7 Serum biochemical parameters of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The results of the effect of administering varying levels of ethanolic extract of ripe pawpaw seeds (EERPS) on the serum biochemical parameters of broiler chickens are presented in Table 4.7. The results showed that varying levels of EERPS to broiler chickens had significant effect ($P<0.05$) on alanine aminotransferase (ALT) and alkaline phosphatase (ALP) while aspartate aminotransferase (AST), creatinine, total protein (TP), and albumin (Alb) were not influenced ($P>0.05$) upon administration of EERPS to the birds.

Table 4.6: Haematological parameters of broilers chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

Parameters	T1	T2	T3	T4	T5	*N R	SEM	P-V	LS
RBC ($x10^{12}$/l)	3.99^{a}	3.26^{c}	3.73^{b}	4.01^{a}	3.98^{a}	1.58 - 4.1	0.08	0.01	*
WBC ($x10^{12}$/l)	22.83	22.83	23.60	23.53	23.30	9.2 - 31.0	0.13	0.40	NS
Hb (g/dl)	9.23	8.92	9.52	8.82	9.67	7.4 - 13.1	0.14	0.24	NS
PCV (%)	27.89	26.33	29.06	26.61	29.09	24.9 -45.2	0.46	0.16	NS

* N R= Normal range (Source = Niger State Veterinary Hospital)

[ab]Means on the same row having different superscripts are significantly different ($P<0.05$), * = Significant, NS = Not significant, SEM = Standard error of mean, LS = Level of Significance, T = Treatment, T = Treatment, T1 = Amprolium, T2 = 2 g/ litre, T3 = 3 g/ litre, T4 = 4 g/ litre, T5 = 5 g/ litre, RBC: Red blood cell, WBC: White blood cell, Hb: Haemoglobin concentration, PCV: Packed cell volume,

Table 4.7: Serum biochemical parameters of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya)* seeds

Parameter	T1	T2	T3	T4	T5	*N R	SEM	P-V	LS
ALT (iu/l)	36.11^{a}	26.22^{b}	27.22^{b}	29.89^{ab}	30.22^{ab}	10 - 36	1.22	0.04	*
AST (iu/l)	4.67	4.00	4.44	4.89	4.11	4 - 20	0.14	0.25	NS
ALP (iu/l)	19.89^{a}	17.67^{ab}	15.33^{b}	17.56^{ab}	18.33^{ab}	10 - 45	0.58	0.04	*
Creat(mmol/l)	4.52	3.56	3.56	3.56	3.67	0 -11	0.19	0.43	NS
TP(mmol/l)	3.79	4.16	4.20	4.29	4.14	2.8-10.1	0.17	0.9	NS
Alb(mmol/l)	1.73	1.89	1.69	1.87	1.87	1.3 -6.4	0.66	0.83	NS

* N R= Normal range (Source = Niger State Veterinary Hospital)

^{ab}Means on the same row having different superscripts are significantly different ($P<0.05$), * = Significant, NS = Not significant, SEM = Standard Error of Mean, LS = Level of Significance, T = Treatment, T = Treatment, T1 = Amprolium, T2 = 2 g/ litre, T3 = 3 g/ litre, T4 = 4 g/ litre, T5 = 5 g/ litre, ALT = Alanine aminotransferase, AST =Aspartate aminotransferase, ALP =Alkaline phosphatase, Creat = Creatinine, TP = Total protein, Alb. = Albumin, P-V= Probability value

4.1.8 Histopathology of the GIT of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The results of the effect of administering varying levels of ethanolic extract of ripe pawpaw seeds (EERPS) on the histology of the gastrointestinal tract of broiler chickens showed that varying levels of EERPS to broiler chickens had histopathological effect on birds as shown in Plate 4.1(control), Plate 4.2 (2g), Plate 4.3 (3g) and Plate 4.4 (4g). However, there was improvement in birds shown in Plate 4.5 (5g) as mild necrosis of glandular tissue and moderate inflammatory cells was observed (H & E, mag x 100) compared to birds in plate 4.2 (2g), plate 4.3 (3g) and plate 4.4 (4g) that had severe necrosis and degeneration of glandular epithelial cells with no inflammatory cell (H & E, mag x 100) seen. However, Plate 4.1(control) had better improvement as unremarkable gastrointestinal glands with inflammatory cells (HaE, mag x 100) were seen.

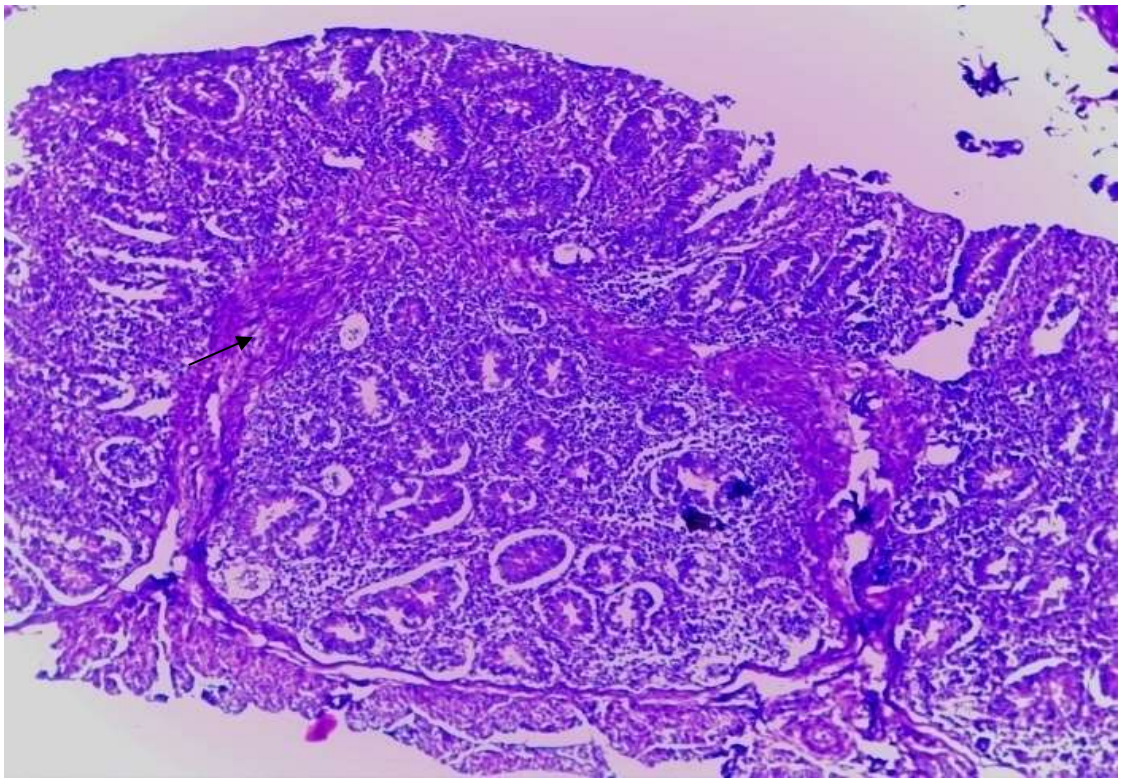

Plate 4.1: Photomicrograph showing remarkable gastrointestinal glands with no inflammatory cells in gastrointestinal tract (GIT) of broiler chicken administered anticoccidial drug (control) (H & E, mag x 100).

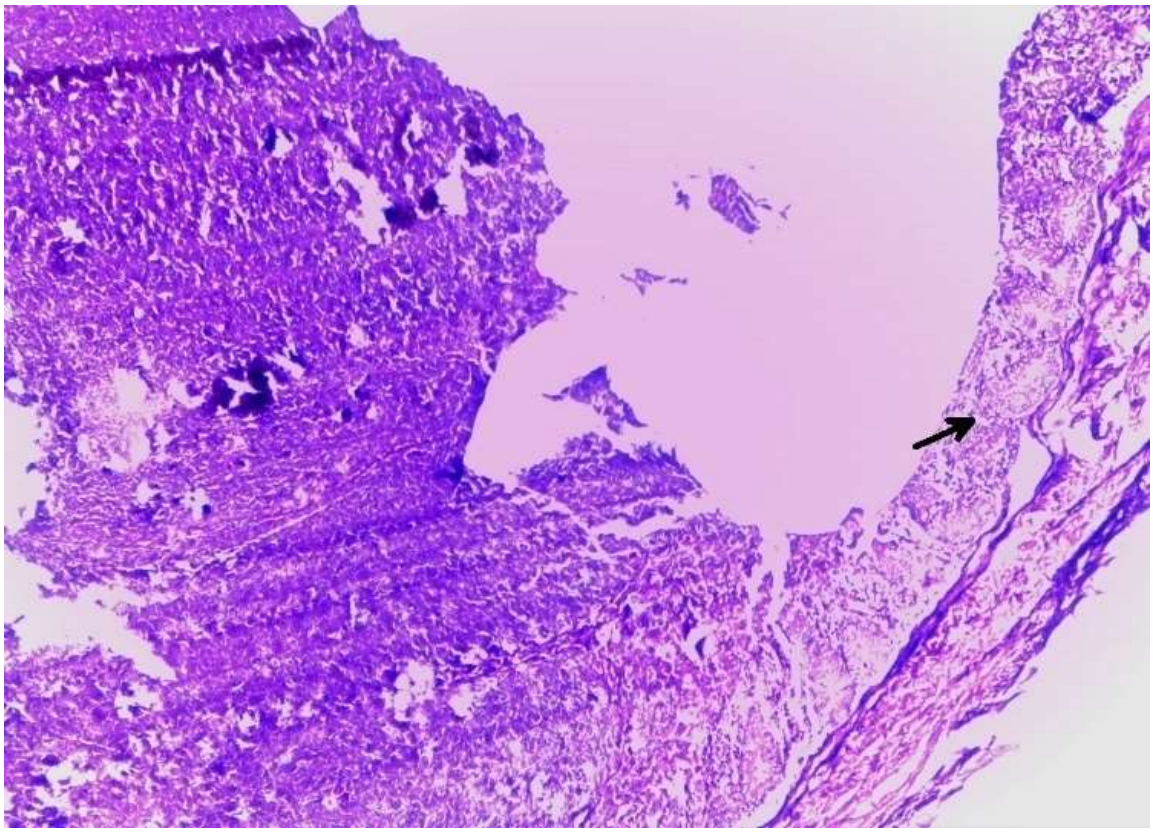

Plate 4.2: Photomicrograph showing areas of severe necrosis and degeneration of glandular epithelial cells with infiltration of neutrophils in the gastrointestinal tract (GIT) of broiler chicken administered 2 g of EERPS (H & E mag x 100).

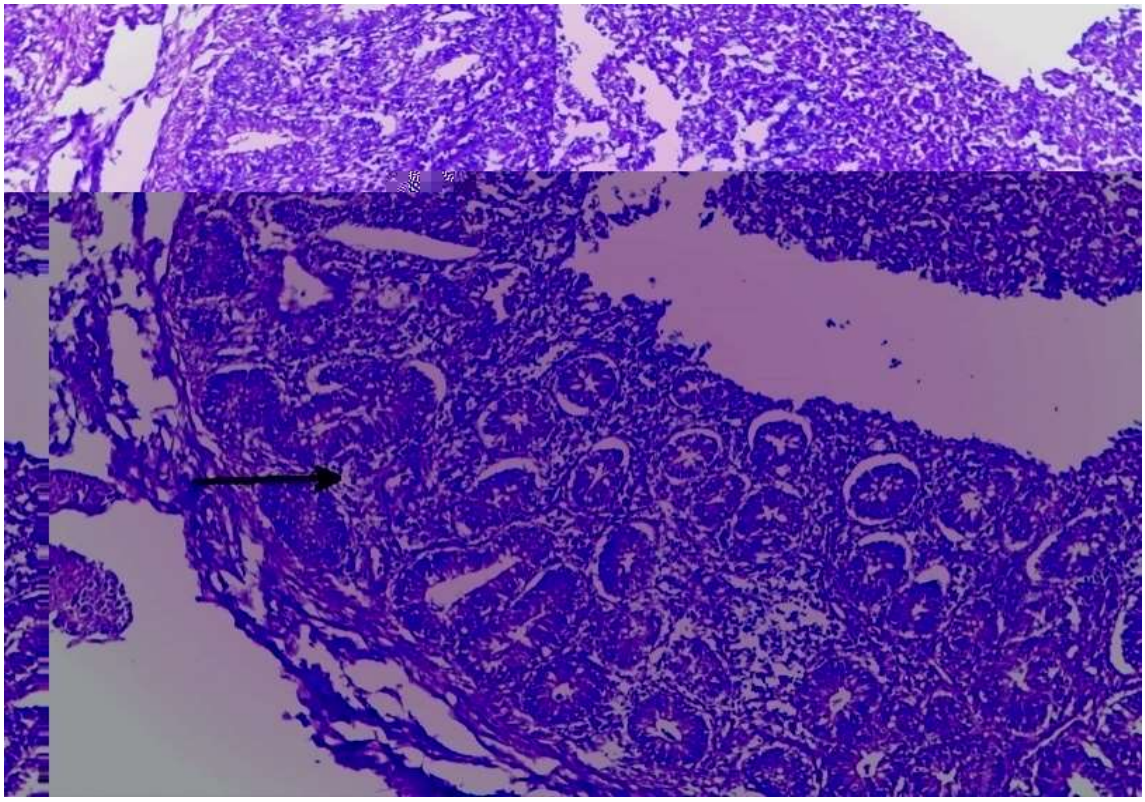

Plate 4.3: Photomicrograph showing areas of severe necrosis, haemorrhage and degeneration of glandular epithelial cells with no inflammatory cell of gastrointestinal tract (GIT) of broiler chicken administered 3 g of EERPS (H & E, mag x 100).

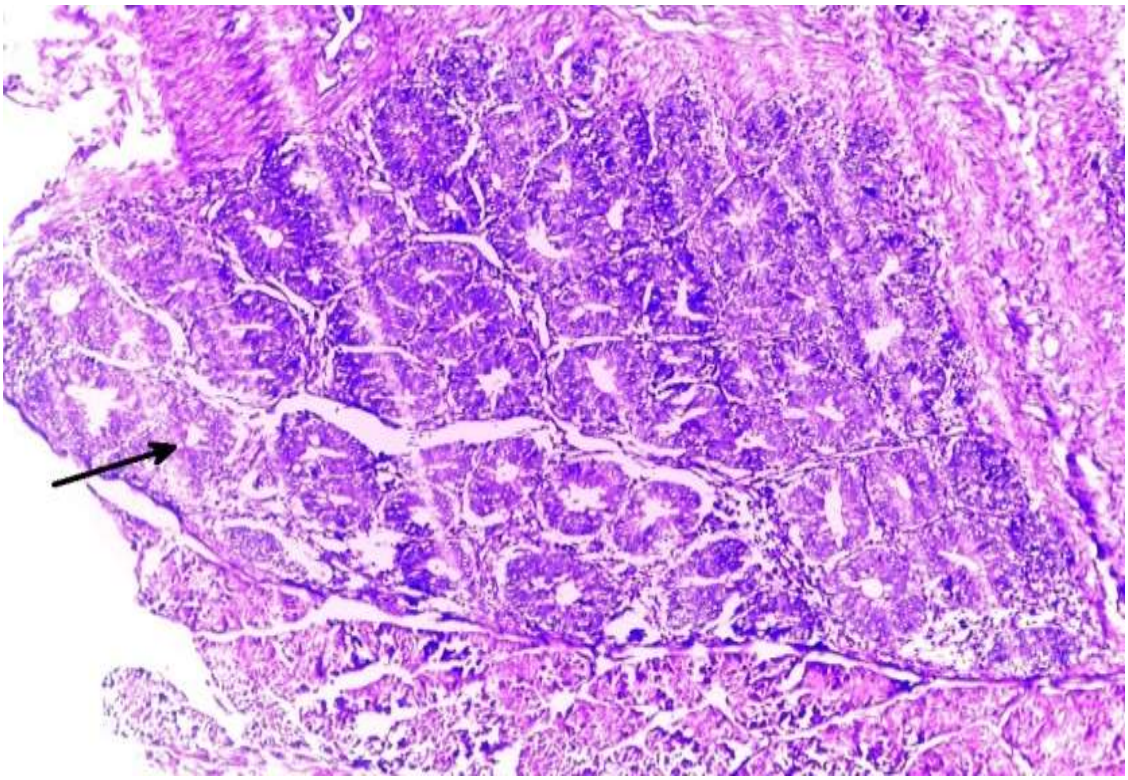

Plate 4.4: Photomicrograph showing areas of necrosis and degeneration of glandular epithelial cells of gastrointestinal tract (GIT) of broiler chicken administered 4 g of EERPS (H & E, mag x 100).

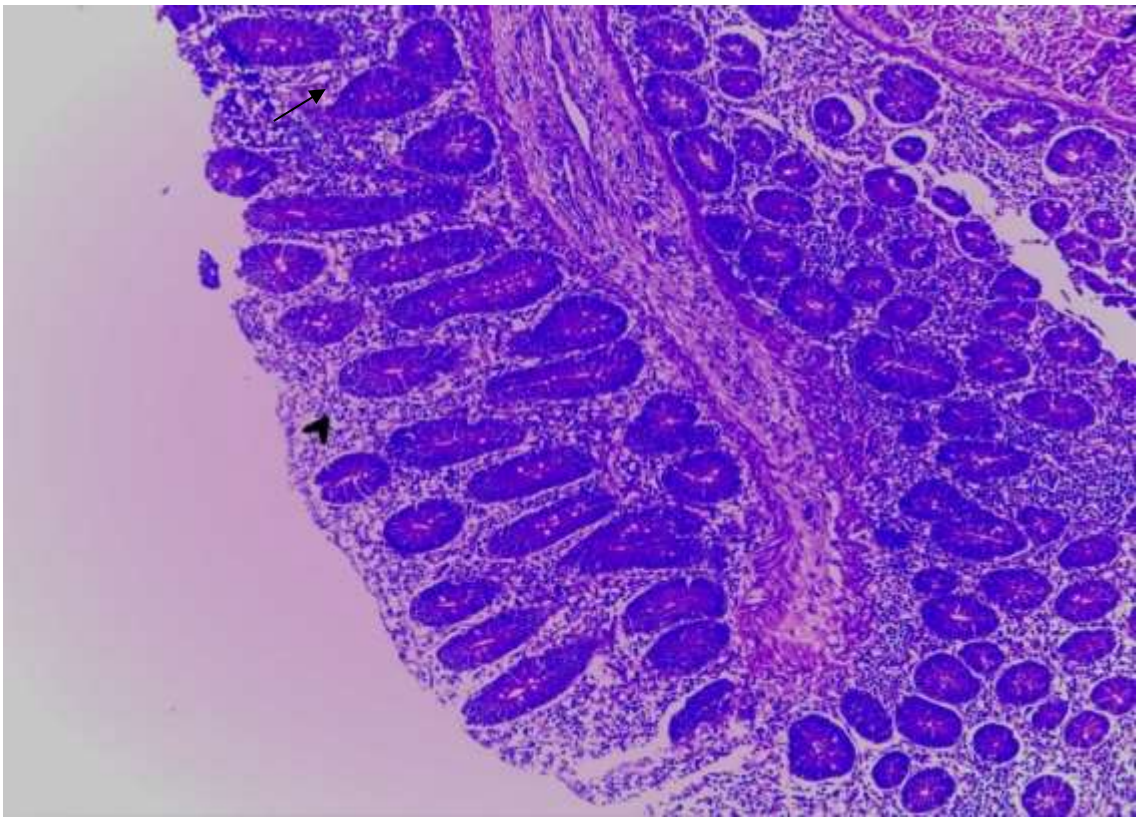

Plate 4.5: Photomicrograph showing areas of mild necrosis of glandular tissue and moderate inflammatory cells of gastrointestinal tract (GIT) of broiler chicken administered 5 g of EERPS (H & E mag x 100).

4.2 Discussion

4.2.1 Proximate composition of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The ethanolic extract of ripe pawpaw (*Carica papaya*) seeds (EERPS) revealed the presence of high percentage of moisture content, ash content, crude protein, ether extract (fat) and nitrogen free extract. The low level of protein in this present study was similar to the findings of Oyeleke *et al*. (2013) who reported low level of protein (5.90 %, 6.00 % and 6.50 %) in matured unriped, ripe and overripe stage, respectively. However, these results disagreed with the findings of Makanjuola and Makanjuola (2018) and Kanadi *et al*. (2021) who reported higher protein content compared to this study. The variations obtained could be attributed to the geographical location of the plants, methods of processing, as well as the season in which the seeds were collected. However, the high presence of nitrogen free extract (NFE) revealed that ethanolic extract of ripe pawpaw seed could serve as a good source of energy because NFE are known to be the main source of energy for organisms including animals such as poultry.

4.2.2 Phytochemical composition of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The ethanolic extract of ripe pawpaw (*Carica papaya*) seeds (EERPS) revealed the presence of phytochemicals such as phenols, flavonoids, saponins, alkaloids and tannins. The highest content of the phytochemicals detected were phenols and saponins. However, phytochemicals such as phenols, flavonoids, saponins and alkaloids have great health potentials which include, antioxidant, anti-inflammatory, antiviral, antifungal, antibacterial, antihelminthic, anti-allergenic, combat neurodegenerative diseases, anticoagulant and immunomodulatory properties (Shewangzaw and Aschalew, 2016 and

Mathew *et al*., 2018). The high presence of tannin value was contrary to the report of El-safy *et al*. (2012) who detected lower value (10.6 mg/ 100 g) in *Carica papaya* seeds. The reasons for the variation could be attributed to multitudes of factors which includes; age of the seeds, differences in the methods of processing, variation in the environmental conditions as well as variation in the genetic make-up and species of *Carica papaya* seeds used for this study. However, the high concentration level of anti-nutritional factor detected such as tannins might have been neutralized due to the potential health benefits of phenols, flavonoids, saponins and alkaloids by reducing free radical formation and scavenging free radicals for optimum production.

4.2.3 Proximate composition of the experimental diets

The proximate composition of the experimental diets used in this study met the nutrient requirement of the starter (energy – 3168.90kcal/ kg and protein – 23.61 %) and finisher (energy – 3279.85kcal/ kg and protein – 20.48 %) broiler chicken diet as stated by NRC (1994).

4.2.4 *In vitro* treatment of coccidial organisms using ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The *in vitro* treatment of coccidial organisms administered ethanolic extract of ripe Pawpaw (*Carica papaya*) seeds (EERPS) shows that, the sporozoites recovery rates varies at different concentrations of ripe pawpaw seed extract which showed EERPS effect on a dose dependent basis. The lowest recovery rate was observed at 50 mg/ml with the sporozoites recovery rate of 24.6 %. The ability of the extract to inhibit sporozoites growth and propagation could be due to the ability of the extract concentration to lyse the cyst thereby forcing the oocyst to empty its cytoplasmic material. This is in agreement with the work of Udo and Abba (2018) who reported the *in-vitro* anticoccidial efficacy *of*

Allium sativum and *Carica papaya* extract at a concentration of 2.5, 5.0 and 10 g/l of distilled water when 4800 unsporulated but viable oocysts were inoculated at room temperature in the laboratory. Highest efficacy at 48 hours with the highest number of unsporulated oocysts were seen at concentration 10 g at 68 % yield for aqueous *Carica papaya* and at 65 % for powder *Carica papaya*. This work is also in agreement with the findings of Yamssi *et al*. (2017) who reported a similar trend when methanolic extract of *Psidium guajava* (guava) were applied on oocyst at 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ ml and 30 mg/ml in *in vitro* experiment. The highest efficacy was 88.67 ± 2.52 % at the concentration of 30 mg/ ml against *E. intestinalis*. The anticoccidial effect of the extract on the sporozoites can also be attributed to its ability to prevent the enzymatic process of the oocysts to sporulate.

4.2.5 Growth performance of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The growth performance of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds (EERPS) indicated that all the parameters had no effect except the final body weight. This could be associated with the improvement of organoleptic characteristics of *C. papaya* seeds administered which increased feed intake and thereby growth performance of poultry (Muazu and Aliyu-Paiko, 2020). This result agrees with the work of Banjoko *et al*. (2020) when varying levels of *Carica papaya* leaf as an anticoccidial were administered to broiler chickens at the rate of 200, 400 and 600 g/ 100 kg of feed and reported effectiveness of *C. papaya* leaf on the final body weight. As the levels of EERPS increases, it was observed that the final body weight increased. These findings could be due to improvement in response to higher levels of pawpaw seeds administered to broiler chickens as reported by Nghonjuyi *et al*. (2020) who worked on varying levels of *Carica papaya* seed extract on Kabir chicks at the rate

of 480, 960, and 320 mg/ kg and discovered that administration of *C. papaya* seed extract improved their body weight gain. The result could also in line with that of Nideou *et al*. (2017) who reported improvement in daily body weight gain of pullet when *C. papaya* seeds at the rate of 0.5, 1.0 and 2 % were included in the rations. The improvement of gastro-intestinal condition and thus increased digestibility and utilization of nutrients could improve the production performance in poultry (Nideou *et al*., 2017).

The feed intake (FI) of the broiler chickens was significantly improved upon increase of EERPS. This could be due to the anthelminthic and antiparasitic activities of *C. papaya* seeds that were likely to have improved the gastrointestinal ecology and improve the health conditions of the birds (Kadiri *et al*., 2016).

The feed conversion ratio (FCR) of the broiler chickens were significantly improved upon increase in EERPS. This could be attributed to the impact of body weight gain and feed intake since, FCR is the ratio of the body weight gain and feed intake. This result is in agreement with that of Nideou *et al*. (2020) who worked on the effect of pawpaw (*Carica papaya*) seed diets on production performance of broiler breeders and hatching parameters at 5 % *C. papaya* seed meal in diets increased feed conversion ratio of broilers breeders. Similarly, Nideou *et al*. (2017) also reported that inclusion levels at the rate of 0.5, 1.0, and 2 % of *C. papaya* seeds in the rations resulted in better FCR than the control groups, in pullet chickens.

The mortality of the broiler chickens varies across the treament groups. As the level of EERPS increases, the survivability of the birds increases. However, birds in T1 (administered anticoccidial drug, Amprolium) had the best survival rate, followed by birds in T5 (5g). The antimicrobial, anthelminthic and antiparasitic activities of *C. papaya* seeds could most likely have improved the health conditions of the birds (Kadiri *et al*.,

2016). This result agrees with the work of Banjoko *et al*. (2020) who reported the evaluation of varying levels of *Carica papaya* leaf as an anticoccidial for broiler chickens at the rate of 200, 400 and 600 g/ 100 kg of feed and discovered that administration of *Carica papaya* leaf meal improve the bird's survivability. The anti-inflammatory properties of *C. papaya* seed in caecal epithelial cell could be detrimental to coccidial reproductive activities (Dakpogan *et al*., 2019).

The oocysts count of coccidial organisms in the faecal samples of broiler chickens were significantly varied on a dose dependent manner. Broiler chickens on T1 (control) had the lowest egg count rate. However, there were decreasing egg count rate with increasing rate of EERPS across the treatment group. This could be as a result of the anticoccial activities of *C. papaya* seeds such as the presence of alkaloids, flavonoids, steroids, saponins, papain and terpenoids possessing antiparasitic activities (Masfufatun *et al*., 2019). This result is in line with the work of Banjoko *et al*. (2020) when varying levels of *Carica papaya* leaf as an anticoccidial for broiler chickens were administered at the rate of 200, 400 and 600 g and reported effectiveness of *C. papaya* leaf in oocysts reduction. Similarly, Nghonjuyi *et al.* (2020) also reported that pawpaw (*C. papaya*) seed extract at the rate of 480, 960 and 3200 mg/ kg body weight reduced faecal egg of *Ascaridia galli* in Kabir chicks in Cameroon. The presence of benyl isothiocyanate in *C. papaya* seed could be responsible for mitochondrial dysfunction of the parasites in chickens (Zhang and Chen, 2017).

4.2.6 Haematological parameters of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The haematological values of RBC were significantly affected upon administration of EERPS treatment ($P<0.05$). However, broiler chickens in all the treatment group tends to have improved haematological competence. However, increase in the administration of

EERPS had no effect in the values of RBC. This indicates that 3 g of EERPS was adequate for this parameter as higher doses of EERPS could lead to reduction in RBC and WBC values but within normal range in the blood of broiler chicken. This could be attributed to the presence of abundant vitamins and minerals in *C. papaya* seeds that had effect on the birds. These results is similar to the findings of Bolu *et al*. (2009) who reported significant difference in RBC values of broiler chickens fed dried pawpaw seeds meal at the rate of 5, 10 and 15 %. However, as the levels of EERPS administration increases across the treatment groups, there were higher WBC, Hb and PCV. The optimum values of RBC, WBC, Hb and PCV indicates the chemical and nutritional attributes of pawpaw seeds as it is reported to be rich in minerals, which are useful in the coagulation of blood, proper functioning of the heart and nervous system, purifies the blood and normal contraction of the muscles (Atta, 1999). This is in line with the findings of Agboola *et al*. (2018) who reported highest values in RBC, PCV and Hb when pawpaw leaf meal was fed to broiler chicken under normal and subnormal diets. Bounous and Stedman (2000) recorded normal haematological parameters of PCV (30 – 40 %), Hb (7 – 13 g/ dl), RBC (3.4 – 4.6 x 10^3 ul) and WBC (12 – 30 x 10^3 ul) in broiler chickens. WBC and PCV obtained in this study were lower than the normal reference values reported by the author. Furthermore, Banjoko *et al*. (2020) also recorded normal hematological parameters of PCV (24.90 – 45.20 %), Hb (7.40 – 13.10 g/dl), RBC (1.58 – 4.10 x 10^{12}/ l) and WBC (9.20 – 31.00 x 10^{12}/ l). The reasons for these variations might depend on multitude of factors among which includes the fact that most normal reference values were established in temperate countries, whose data may not effectively reflect tropical animal characteristics due to differences in environmental conditions as well as variations in the genetic make-up of the broiler chicken used for the study (Onunkwo *et al*., 2018).

4.2.7 Serum biochemical parameters of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The serum biochemical indices of broiler chicken fed varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds indicated that ALT, and ALP were significantly influenced ($P<0.05$) while AST, Creatinine, total protein, and albumin were not significant influenced ($P>0.05$) on administration of EERPS to the birds. These results disagree with the findings of Umar and Muhammed (2020) who reported insignificant difference in ALT and ALP values in the blood of broiler chickens when fed graded levels of *C. papaya* seed powder at 0, 0.50 and 1 %. However, this result agrees with the findings of Umar and Muhammed (2020) who reported insignificant difference in AST, total protein, albumin and creatinine values in the blood of broiler chickens when fed graded levels of *C. papaya* seed powder (at 0, 0.50 and 1 %). On the contrary, Bolu *et al*. (2009) reported significant difference in the values obtained in creatinine, total protein and albumin when dried pawpaw seed at the rate of 0, 5, 10 and 15 % were included in the diets of broiler chickens. Similarly, Henry *et al*. (2017) also reported no significant difference on total protein, urea, albumin, globulin when weaned rabbits were fed pawpaw leaves as feed supplement (as fresh and as wilted pawpaw leaves). Niger State veterinary hospital reported normal range values of biochemical indices of ALT (10.0 – 120.0 iu/l), AST (4.0 – 20.0 iu/l), ALP (10.0 - 45.0 iu/l), Creatinine (0 – 11.0 mmol/l), total protein (2.8 - 10.1 mmol/l) and albumin (1.3 - 6.4 mmol/l). This variation might be as a result of differences in the methods of processing of pawpaw plant parts as well as doses administered to the birds. The significant values observed in ALT and ALP are statistically similar among treatment groups on administration of EERPS and the control group. These are indication of normal liver and kidney function that was not threatened

by addition of EERPS. High creatinine values are a measure of especially muscle amino acid degradation and early pointer to depressed liver and kidney functions (Wards *et al*., 1985). Creatinine values of broiler chicken administered EERPS were numerically lower but not significant (P>0.05) compared to the control. Elevated value in the control may suggest deflection of tissue creatinine phosphate and this may adversely affect the muscles mass (Johnson and Giulivi, 2005). This could imply that there was slightly better protein metabolism and utilization in the treatment groups than the control. Therefore, EERPS can be administered to broiler chickens to improve performance.

4.2.8 Histopathology of the gastrointestinal tract (GIT) of broiler chickens administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds

The histological changes of gastrointestinal tract of broiler chicken administered varying levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seeds indicated improvement of the GIT at 5 g/litre of drinking water though less improvement was seen compared to the control (Amprolium). The histological improvement of the GIT on administration of EERPS to the birds could be due to several bioactive components suggested to play a vital role in immunomodulatory and anti-inflammatory activities present in *C. papaya* seed. This result is in agreement with the work of Oloruntola (2019) who reported that there was marked proliferation of polymorphonuclear and mononuclear cells, activation of liver microphages system and the kupffer cells in the liver of broiler chickens when fed pawpaw leaf and seed meal composite mix dietary supplementation. Pawpaw (*C. papaya*) seeds extract has been revealed at the concentration of 10 mg/ ml to inhibit the expression of interferon-gamma, tumor necrosis factor-alpha, interleukin-6, and nuclear factor-κB in pancreatic (HPDE-6) epithelial cells stimulated with methyl isocyanate (Pathak *et al*., 2014). On the contrary, Omidiwura *et al*. (2020) reported eroded villi of mucosa layer

when *C. papaya* leaf meal diet at 3 % and combination of 1.5 % *C. papaya* and 1.5 % *Chromolaena odorata* leaf meal diet showed severe infiltration of inflammatory cells in the gut of broiler chicken. However, Kadiri *et al*. (2016) suggested that enzymes, vitamins, polyphenols and other active components are attributed to the pharmacological and immune-modulatory properties of *C. papaya* seeds.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the result obtained from this study, it can be concluded that ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) at 50 mg/ml concentration produced reduction in the number of sporozoites rate in *in vitro* experiment.

Ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) administered to broiler chickens improved their final body weight and body weight gain at the rate of 5 g/ litre of drinking water without any deleterious effect on their performance.

Ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) proved viable and effective in the treatment and inhibition of coccidial organisms in broiler chickens. There was reduction in oocysts count upon increase in the levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seed administered to broiler chickens.

The survival of the broiler chickens was improved upon increase in levels of ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) at 5 g/litre, administered to broiler chickens.

The haematological and biochemical profiles were maintained within the normal range of values in broiler chickens administered EERPS. The administration of EERPS (at 5 g/litre) improved the histopathology of the gastrointestinal tract of the broiler chickens in Treatment 5.

5.2 Recommendations

i. Ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) at 5 g/ litre of drinking water could be administered to broiler chickens to boost poultry production without any deleterious effect on the birds and it consumers.

ii. The use of EERPS in poultry production should be encouraged against the use of synthetic anticoccidial drugs (such as amprolium) in order to avoid health challenges arising from drug residues and resistance in poultry production.

iii. Ethanolic extract of ripe pawpaw (*Carica papaya*) seed (EERPS) could be given daily other than weekly for improvement in poultry production.

5.3 Contribution to Knowledge

It was discovered that the oocysts count of coccidial organisms in the faecal samples of broiler chickens were significantly reduced upon increase in the administration of ethanolic extract of ripe pawpaw (*Carica papaya*) seed across the treatment groups on a dose dependent basis. Treatment 5 (T5) had the lowest oocyst count among the broiler chickens administered ethanolic extract of ripe pawpaw (*Carica papaya*) seed treatment groups. The egg count rate of (T5) decreased from intial oocytes count of 42.89% to 14.17% as final oocyst count. This result has established the anticoccial activities of ethanolic extract of ripe pawpaw seeds.

Additionally, the study revealed the potency of the in vitro treatment of coccidial organisms administered ethanolic extract of ripe pawpaw seeds (EERPS). It was observed from results that the effect of the administration of different concentrations of ripe pawpaw seed extract was dose dependent on the sporozoites recovery rates. The best result was obtained in Treatment 5 (T5) with the lowest sporozoite recovery rate of 24.6 % when compared with the control which recorded the highest sporozoite recovery of

57.10%. This remarkable achievement was due to the ability of the extract to inhibit sporozoites growth and propagation. In this study from the result obtained it was revealed that the administration of ethanolic extract of ripe pawpaw seeds up to 5 g/ litre to drinking water boost poultry production without any deleterious effect on growth performance, blood haematology, biochemical profile and histopathology of the birds. This implies that ethanolic extract of ripe pawpaw seeds could help in the control of coccidiosis and in overall improvement in poultry production.

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