

**EFFICACY OF AQUEOUS EXTRACT OF *XYLOPIA AETHIOPICA* WHOLE FRUITS  
ON EXPERIMENTAL *ASCARIDIA GALLI* INFECTION IN GUINEA FOWL KEETS  
(*NUMIDA MELEAGRIS GALEATA PALAS*)**

**BY**

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ZARIA**

**AUGUST, 2016**

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**DEPARTMENT OF VETERINARY MEDICINE,  
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ZARIA**

**AUGUST, 2016**

## DECLARATION

I declare that the work in this Dissertation entitled “Efficacy of Aqueous Extract of *Xylopi aethiopica* Whole Fruits on Experimental *Ascaridia galli* Infection in Guinea Fowl Keets (*Numida meleagris galeata* Palas)” has been performed by me in the Department of Veterinary Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other Institution.

Nizam Ikira MUSTAPHA .....  
Signature Date

## CERTIFICATION

This Dissertation entitled **“EFFICACY OF AQUEOUS EXTRACT OF *XYLOPIA AETHIOPICA* WHOLE FRUITS ON EXPERIMENTAL *ASCARIDIA GALLI* INFECTION IN GUINEA FOWL KEETS (*NUMIDA MELEAGRIS GALEATAPALAS*)”** by Ikira Nizam MUSTAPHA meets the regulations governing the award of the degree of Master of Science of Ahamadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## ABSTRACT

The major control strategy adopted against helminth parasites in Nigeria is the use of conventional anthelmintics. However, the high cost of modern anthelmintics has limited their use in rural areas, coupled with the emergence of resistant strains of pathogenic helminthes. It was against this background that the desire to search for alternative additional chemotherapeutic agents that this study was initiated; to evaluate the effects of *Xylopia aethiopica* (*Xa*) whole fruit extract on anthelmintic efficacy, haematological and biochemical parameters in guinea fowl keets experimentally infected with *Ascaridia galli*. One hundred guinea fowl keets were randomly assigned to five groups (I, II, III, IV and V) of 20 birds each. Each Keet in groups I, II, III, and IV was inoculated with 700 infective *A. galli* eggs contained in 0.4 ml normal saline, while keets in group V were uninfected and untreated. Before administration, toxicity study was conducted on the *Xa* fruit extract. At 3 weeks post-infection and 3 days after first detection of *A. galli*, keets in groups I and II were treated with 2,000 mg/litre and 4,000 mg/litre of *Xa*, respectively, while those in group III were treated with 1,000mg/litre of piperazine (as reference standard) for three days. The efficacy of the *Xa* and piperazine were determined based on percentage deparasitization (postmortem worm count). Blood samples were collected through the wing vein of three keets from each group for haematological and serum biochemical analyses. Packed cell volume (PCV), haemoglobin concentration (Hb)and erythrocyte count were determined by the microhaematocrit, cyanmethaemoglobin and haemocytometry methods respectively. Serum biochemical assay was carried out for Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST)and total serum albumin (ALB)on samples from all groups. The LD<sub>50</sub> of the *Xa* extract was above 5000 mg/kg. There was significant difference ( $p<0.05$ ) in the egg per gram count between all groups post treatment. The percentage

deparasitization observed in groups I, II and III were 25.5%, 44.4% and 100%, respectively. Increases in PCV and Hb concentration were observed post-treatment with aqueous extract of *Xa* whole fruit in *A. galli*-infected keets in groups I ( $38.7 \pm 1.25$  %) and II ( $38.2 \pm 1.03$  %) when compared to infected/untreated keets in group IV ( $34.3 \pm 3.42$  %). Decreases in serum aspartate aminotransferase and alanine aminotransferase levels were also observed post-treatment of keets in *A. galli*-infected groups I ( $67.2 \pm 7.12$  u/l and  $3.8 \pm 0.37$  u/l) and II ( $74.0 \pm 9.13$  u/l and  $3.6 \pm 0.24$  u/l) when compared to the values in infected/untreated keets in group IV ( $81.6 \pm 4.76$  u/l and  $4.4 \pm 0.87$  u/l), respectively. It was concluded that the *XA* whole fruit aqueous extract used in this study has a dose-dependent anthelmintic effect on *A. galli* in guinea fowls and was able to reduce the severity of the effect of *A. galli* infection on the haematological and serum biochemical parameters. The use of aqueous extract of *Xylopi* *aethiopica* whole fruits as an anthelmintic remedy especially in rural poultry is recommended.

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## **LIST OF ABBREVIATIONS**

ABU	– Ahmadu Bello University
ALB	– Albumin
ALP	– Alanine Phosphatase
ALT	– Alanine Transaminase
ANOVA	– Analysis of Variance
AST	– Aspartate Transaminase
D	– Days
DLC	- Differential Leucocyte Count
EDTA	– Ethylenediaminetetracetic acid
EPG	– Egg per gram
FeCl <sub>3</sub>	- Iron (III) Chloride
FEC	– Faecal egg count
GGT	- Gamma Glutamyl Transpeptidase
g/dl	- grams per decilitre
gm	- Gram
h	– hour(s)
Hb	– Haemoglobin
H <sub>2</sub> SO <sub>4</sub>	- Sulfuric Acid
Kg	- Kilogram
L	- Litre
LD	– Lethal dose
M	- Mole

Mg - Milligram  
Mg/l - Milligram per litre  
Ml - Millilitre  
NaOH - Sodium Hydroxide  
nm- Nanometer  
PCV – Pack Cell Volume  
PP – Plasma Protein  
RBC – Red Blood Cell  
SEM – Standard error of mean  
TP – Total Protein  
µl - Microlitre  
U/L - Unit per Litre  
WBC – White Blood Cells  
*Xa* - *Xylopi aethiopica*

## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **1.1 Study Background**

The guinea fowl (*Numida meleagris*) is a common indigenous bird of the African continent. In the northern part of Nigeria, these birds are kept in most villages and are abundant in the wild. There are approximately 44 million guinea fowls in captivity in the country, and the products (meat and eggs) from these birds are well accepted socially (Ayeni and Ayanda, 1982). According to Mareko *et al* (2008), guinea fowls originated in Africa where they still retain many of their original traits.

Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans (Adang *et al.*, 2010).

The major control strategy adopted against helminth parasites in Nigeria is the use of anthelmintics (Ibrahim *et al.*, 1983). However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases, widespread intensive use of sometimes low-quality anthelmintics has led to development of resistance and hence a reduction in the usefulness of available anthelmintics (Waller, 1997a; Monteiro *et al.*, 1998). Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites, and also as a means of reducing the cost of controlling helminthic diseases (Kelly and Hall, 1979; Okon *et al.*, 1980; Taylor and Hunt, 1989; Coles and Roush, 1992), the emergence of resistant strains of pathogenic helminths has stimulated the desire to

search for additional chemotherapeutic agents that might allow more efficient control of helminth parasites (Hammond *et al.*, 1997; Waller, 1997b). A practical solution to this is to develop effective drugs from reasonably less expensive and available raw materials. This can rationally be approached through the study of indigenous traditional plant remedies. In Nigeria, herbal treatment of helminthosis is widely practised by herbalists and the nomadic Fulanis who are the major cattle rearers in Nigeria (Nwude and Ibrahim, 1980). Plants and their derivatives play a key role in health and have long been known to possess biological activity (Abass, 2012). It is a known fact that thirty per cent of all modern drugs are derived from plants and available evidence suggests that approximately 80% of Africans rely on traditional healthcare practitioners and medicinal plants for their daily healthcare needs (Johnson *et al.*, 2007; McKay and Blumberg, 2007).

*Xylopia aethiopica*, commonly known as “African guinea pepper” or “Ethiopian pepper” is an angiosperm of the Annonaceae Family, and grows predominantly in humid forest zones of West Africa (Puri and Talata, 1978; Woode *et al.*, 2011). In Nigeria, it is found all over the lowland rain forest and most fringe forest in the savannah zones (Sofowara, 1978). It is used as a pepper substitute in Europe and India (Sofowara, 1978) and highly valued in other countries because of its medicinal and pharmacological properties (Okeke *et al.*, 2008).

Suleiman *et al.* (2005) reported the anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. However, little or none is known about its anthelmintic activity in poultry, hence this work was designed to evaluate the efficacy of

aqueous extract of *Xylopi aethiopica* fruits on experimental *Ascaridia galli* infection in guinea fowl keets.

## **1.2 Statement of Research Problem**

*Ascaridia galli* is a common parasite of poultry and has been reported in chicken, turkey, guinea fowls, pigeons, duck, and goose (Ruff and Norton, 1997). It has been reported as a common parasite of pigeons and doves in Zaria (Abdullahi *et al.*, 1992; Oniye *et al.*, 2000; Audu *et al.*, 2004; Gadzama *et al.*, 2005). Ascariidiasis is an intricate problem to poultry breeders, and so it could be to guinea fowl breeders and fanciers. It is one of the major causes for reduction in egg production, reduced growth rate in broilers, and consequently, responsible for economic losses to the poultry industry (Adang *et al.*, 2010).

*Xylopi aethiopica* is one of the most pungent spices plants that are native to the low land rain forests and moist fringe forests in the Savanna zones and coastal regions of Africa (Okwu, 2001). It has a savory aroma and has been used in Ayurvedic medicine in the treatment of headache, neuralgia, cough, rheumatism, bronchitis, dysentery, biliousness, female sterilization and skin infections. The plant is also used as a carminative, purgative and as an abortifacient (Burkill, 1985). In Nigeria, the major control strategy adopted against helminth parasites is the use of anthelmintics (Ibrahim *et al.*, 1983). However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics (Monteiro *et al.*, 1997) has led to development of resistance and hence a reduction in the usefulness of available anthelmintics (Waller, 1997a). People in the rural settings are also known to keep their poultry birds usually mixed in an extensive system of

management; mostly guinea fowls and chickens. These usually give chance for persistent infestation and cross infestation of the birds with helminthes leading to huge economic losses. Also, the rural dwellers have more access to this plant as such utilize it in different domestic ways.

### 1.3 Justification of the Study

In Africa, especially south of the Sahara, the use of plants and their extracts for the treatment and management of diseases has been in existence since ancient times. Factors such as poverty and illiteracy still militate against availability and accessibility of conventional medical services. A larger number of these tropical plants and their extracts have shown beneficial therapeutic effects, including fertility enhancement, contraceptive, anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac effects (Raji *et al.*, 2006). Among the promising medicinal plants is *Xylopiya aethiopica*, which is an angiosperm of the Annonaceae Family, it is a tropical evergreen tree growing up to 20 metres bearing aromatic fruits and locally known as 'Hweentia' or 'Kimba' (Burkill, 1985). Dry fruits of *Xylopiya aethiopica* are used in Ghana, Nigeria and Cameroon as spices. Fruit decoction of *Xylopiya aethiopica* is used to treat bronchitis, asthma, infertility, arthritis and rheumatism and as postpartum tonic (Burkill, 1985). Different works have been done to explore the diverse usage of this plant in traditional medicine. Suleiman *et al.* (2005) found out that the crude methanol extract of the plant did produce significant anthelmintic activity against *Nippostrongylus brasiliensis* in rats. The result of this study will therefore provide baseline information on the effects of *Xylopiya aethiopica* fruits on anthelmintic efficacy, haematological and biochemical parameters in guinea fowl keets experimentally infected with *Ascaridia galli*.

#### **1.4 Aim of the Study**

The aim of this study was to evaluate the efficacy of aqueous extract of *Xylopi aethiopica* fruits on experimental *Ascaridia galli* infection in guinea fowl keets.

#### **1.5 Objectives of the Study**

The specific objectives of this study were to evaluate the:

1. anthelmintic efficacy of *Xylopi aethiopica* in guinea fowl keets infected with *Ascaridia galli*.
2. effects of *Xylopi aethiopica* on some haematological parameters of guinea fowl keets infected with *Ascaridia galli*.
3. effects of *Xylopi aethiopica* on some serum biochemical parameters of guinea fowl keets infected with *Ascaridia galli*.

#### **1.6 Research Questions**

1. does *Xylopi aethiopica* have anthelmintic effect on *Ascaridia galli* in guinea fowl keets?
2. does *Xylopi aethiopica* alter the haematological parameters of guinea fowls infected with *Ascaridia galli*?
3. does *Xylopi aethiopica* alter the serum biochemical parameters of guinea fowls infected with *Ascaridia galli*?



## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 The Guinea Fowl

##### 2.1.1. General Introduction

The term “Guinea fowl” is the common name of the seven species of gallinaceous birds of the family *Numididae*, which is indigenous to Africa (Payne, 1990; Smith, 1990). It is well adapted to the realities of life on the African continent (Nwagu and Alawa, 1995). The strains are descended from the helmeted guinea fowl, *Numida meleagris*. In many parts of the world, guinea fowls are raised mainly for their gamey flesh and eggs. Guinea fowl has a taste similar to other game birds and has many nutritional qualities that make it a worthwhile addition to the diet (Ensminger, 1980; Feltwell, 1992). The meat of a young guinea fowl is tender and of fine flavour, resembling that of wild game. The meat is lean and rich in essential fatty acids. Guinea fowl has a high yield of 80% after processing, with excellent meat to bone ratio (CAB International, 1987). Other people raise them for their unique ornamental value. Of the three domestic varieties (the Pearl, the White and the Lavender), the purplish-coloured pearl is the most common. The largest member of the family is the 60 cm long vulturine guinea fowl, *Acryllium vulturium*, found in tropical East Africa. They are classified in the Phylum Chordata, subphylum Vertebrata, Class Aves, Order *Galliformes* and Family *Numididae* (Ayeni, 1979). The domesticated guinea fowls are kept for egg and meat production (Smith, 1990; Ajala *et al.*, 1997; Maganga and Haule, 1998).

Meat of the guinea fowl can be distinguished from duck, chicken and turkey meats by its nice gamey taste (Feltwell, 1992). Many hotels and restaurants in large cities around the world serve

guinea fowl meat at banquet and club dinners as a special delicacy or as a substitute for gamey birds, grouse, partridge, quail and pheasant (Ensminger, 1980). The meat of the guinea fowl has higher protein (23% *vs.* 21%), lower fat (4% *vs.* 7%) content and higher edibility after cooking (80% *vs.* 65%) than chicken (CAB International, 1987). Therefore, it is likely to be more appealing to consumers than chicken meat (Feltwell, 1992). The Greeks and Romans are reported to be the first to domesticate guinea fowls (Ayorinde, 1987). Although guinea fowl are thought to have originated in Africa, its production has increased rapidly throughout the entire world (Teye and Gyawu, 2002). Guinea fowl can be successfully reared under semi-intensive conditions with less effort. That is why it has been very important for Third World countries (Ayorinde, 1990). With the help of developments in organic farming techniques in recent years, the Guinea fowl has been given importance as an alternative poultry (Sarica *et al.*, 2003). While partly known in some parts of Asia and South America it is widely bred in some European countries such as France, Italy and Belgium (Embury, 2001; Champagne, 2003).

As a result of the scientific researches carried out in France, fast-growing strains are now in use, for which egg production can reach up to 190 eggs in a year (Muğlalı, 2001). Breeders generally produce well for 2 to 3 years (Ayorinde *et al.*, 1989). While hens can lay down up to 175-200 eggs after a 35-week-laying period, there are lines reaching up to 1.5-1.7 kg of live weight and a ratio of 2.7-2.9 feed conversion within 11-12 weeks by intensive breeding and selection works (Le Coz-Douin, 1992). These fowls have kept their characteristics such as being matured, high reproduction and resistant to diseases under different ambient temperatures (Boko, 2004). Moreki (2009) reported that guinea fowl meat also commands a premium price. Other advantages of rearing guinea fowl include low production costs, greater capacity to utilize green

feeds, control of ticks and other pests and better ability to protect itself against predators (Moreki, 2009). Furthermore, guinea fowl and their eggs are used for scientific research, notably in physiology (Ikani and Dafwang, 2004). These advantages make guinea fowl suitable to the rural areas where commercial chicken production has failed due mainly to high input costs and inadequacies in health management (Ayorinde, 1990).

### 2.1.2 Origin and Distribution

Historical records indicate that the guinea fowl derived its name from the Guinea Coast of West Africa (Roy and Wimberley, 1979; Awotwi, 1987; Smith, 1990; Teye and Gyawu, 2002; Dei and Karbo, 2004) where today some still remain in the wild (Dei and Karbo, 2004). Guinea fowl has ubiquitous distribution in Africa where it has distinct popularity among smallholder farmers (Nwagu and Alawa, 1995). The guinea fowl is a bird that belongs to a group *Carinatae* (flying birds), Order *Galliformes* (includes turkeys, chickens and pheasants), and the Family *Numididae* (that is the guinea fowl of African origin). It belongs to the genus *Numida*. The genus has two species, *Numida ptilorhycha*, that is the blue- wattled guinea fowl and *Numida meleagris* that is red- wattle guinea fowl of West Africa. The wild guinea fowl is native to West Africa but are now kept in many parts of the world (Smith, 1990; Bell and Smith, 2003; Dei and Karbo, 2004).

### 2.1.3 Advantages of Guinea Fowl over Chickens

The guinea fowl is a promising genetic resource for evolving a low-input poultry enterprise mostly in developing countries, and has the potential for reducing poverty (Teye and Gyawu, 2002). Guinea fowls normally cost more than chicken and are therefore a very good source of income for farmers. The income from the sales of the birds is used by the farmers for paying school fees of

their wards, medical bills, buying clothes for wives and children, paying water and energy bills and for buying food in periods of food shortage (Apiiga, 2007). Keeping guinea fowls provide not only more protein but substantial income to peasants, when compared with chicken farming (Idi *et al.*, 2001). The yield of edible meat in guinea fowl is higher than that in chicken due to the slenderness of its skeleton thus, after dressing it yields 80% edible meat as compared with 65% for chicken (Koney, 1993). Sayila (2009) reported that the off-take rate and mortality for guinea fowl in Botswana is only 3.4% and 2.2%, respectively, whereas chickens have scores of 10.6% and 6.8%. This gives guinea fowl, considering the local conditions, a better chance of becoming a favourite in future. In Nigeria, Ikani and Dafwang (2004) reported that guinea fowl eggs command premium prices because of their gamey flavor. In addition, they have better storage ability than chicken eggs, as their eggshell do not crack easily due to their thickness. Guinea fowl eggs shell are thicker than chicken eggs (Yildirim, 2012). The guinea fowl meat contains less cholesterol and fat but higher in protein as compared to the meat of other poultry species, which makes guinea fowls to be sold at high prices (Apiiga, 2007). According to Moreki and Seabo (2012), the guinea fowl's attractive plumage and value as a table bird with game-type flavour and high meat-to-bone ratio has ensured its wide acceptance.

Other advantages of rearing guinea fowl include low production costs, greater capacity to utilize green feeds, control of ticks and other pests and better ability to protect itself against predators (Moreki, 2009). Furthermore, guinea fowl and their eggs are used for scientific research, notably in physiology (Ikani and Dafwang, 2004).

#### 2.1.4 Breeds of Guinea Fowls

Guinea fowl has been classified into four genera (*Agelastes- Phasiadus*, *Guttera*, *Acryllium* and *Numida*), six species and sixteen subspecies (Ayorinde, 2004). There are two main species of guinea fowl in Nigeria, which have been described; the crested guinea fowl (*Guttera edopuardi*) which is found in the rain forest zones and derived savanna and the helmeted guinea fowl (*Numida meleagris* and *Numida ptilorhycha*). The *Numida ptilorhycha* is indigenous to the deciduous rain forest zone of southern Nigeria while *Numida meleagris* is currently domiciled in the Northern part of Nigeria but it is spreading to other smallholder farming areas (Ayorinde, 1987). Guinea fowl *Numida meleagris*, production is associated with smallholder farmers in Africa (Smith, 2000) and is described as a “poor man’s pheasant” (Bonds, 1997). However, the commonly known varieties found in Nigeria are seven, they are namely:

##### 2.1.4.1 Pearl variety

The pearl guinea fowl, one variety or subspecies of the helmeted guinea fowl, is the best known of the guinea fowl bird family, *Numididae*, and the only member of the genus *Numida*. It is found in Africa, mainly south of the Sahara, and has been widely introduced into the West Indies and Southern France (Martinez, 1994). The helmeted guinea fowl is classified as *Numida meleagris*, its western African subspecies as *Numida meleagris galeata* (Ayorinde, 2004). The pearl guinea fowl (*Numida meleagris*) has a bony casque or helmet on top of its head covered with horny cartilage. Each of the nine subspecies of helmeted guinea fowl has a characteristic helmet shape (Martinez, 1994). In contrast to this finding, Ayorinde (2004) argues that there are actually only four distinct subspecies of the helmeted guinea fowl. Each is also characterized by different colouring of the bare parts of the head, wattle and neck feathers, as well as the absence

or presence of conspicuous bristles near the nostrils (Martinez, 1994). They are large birds which measure from 40-71 cm in length, and weigh 700-1600 g (Martinez, 1994). Ayorinde (2004) describes the pearl guinea fowl as round-shouldered, clad in sheer dark feathers with delicate white polka-dots. They are characterized by the presence of a helmet and lateral wattles, and the bare skin of the neck, chin and throat (Awotwi, 1987). Like other guinea fowls, this species has an unfeathered head, in this case decorated with a dull yellow or reddish bony knob, and red and blue patches of skin. The wings are short and rounded, and the tail is also short. Male pearl guinea fowl celebrate the beginning of breeding season with a parade, a single file of birds chasing each other with their heads lowered and their wings raised over humped backs. This ritual is a display of both aggression and courtship (Ayorinde, 2004).

#### 2.1.4.2 The white-breasted variety

The guinea fowl (*Agelastes meleagrides*) is black with a broad white collar, and is considered one of the most endangered species of Africa because of habitat destruction and hunting pressure. The white-breasted guinea fowl is one of the members of the two species of the genus *Agelastes* (Ayorinde, 2004).

#### 2.1.4.3 The lavender variety

This guinea fowl also resembles the pearl variety, but the plumage is light gray or lavender regularly dotted with white markings (Ikani and Dafwang, 2004). The lavender guinea fowls are one of the three variety or subspecies of the helmeted guinea fowl (Ayorinde, 2004; Jacob and Pescatore, 2011).

#### 2.1.4.4 The crested and plumed guinea fowl

The Crested guinea fowl (*Guttera pucherani*) is a member of the *Numididae*, the guinea fowl bird family. It is found in open forest, woodland and forest-savanna mosaics in sub-Saharan Africa. They have slightly curled feathers and the plumage is overall blackish with dense white spots. It has a distinctive black crest on the top of its head, the form of which varies depending upon subspecies. They occur in flocks averaging fewer than 20 birds. During early mornings after descending from their nightly roosts, crested guinea fowl flocks move into forest glades to preen and socialize in the warmth of the morning sun. Crested guinea fowl fly up into trees to feed on fruit rather than eat maize scattered on the ground (Ayorinde, 2004).

#### 2.1.4.5 The plumed variety

The Plumed guinea fowl (*Guttera plumifera*) is a member of the guinea fowl bird family. It is found in humid primary forest in Central Africa. It resembles some subspecies of the Crested guinea fowl, but has a straighter (not curled) and higher crest, and a relatively long wattle on either side of the bill. The bare skin on the face and neck is entirely dull grey-blue (Ayorinde, 2004).

#### 2.1.4.6 The white variety

This variety like the name indicates, has pure white plumage. Its skin is lighter in colour than that of the pearl variety. The pure white colour of this variety is definitely advantageous with respect to minimizing heat stress during the excessive heat period (Ikani and Dafwang, 2004).

#### 2.1.4.7 The vulturine guinea fowl

The Vulturine guinea fowl (*Acryllium vulturinum*) is from the semi-arid regions of east Africa. The vulturine is not commonly raised in the United States as it is more sensitive to cold and lacks the hardiness of the common guinea fowl. It has a helmetless head and resembles the look of a vulture (Jacob and Pescatore, 2011). It is the largest and most ornate species. It has a cape of long hackle feathers extending from the lower neck to the breast. These feathers are white, edged with black and bright cobalt blue. This species may be readily seen in the non-breeding season in flocks of up to 30 individuals. The head of the vulturine guinea fowl is mostly featherless except for a chestnut- brown patch of short feathers on the sides and back of the head. This species has a hackle of spear-shaped feathers which have lengthwise stripes of black, white and blue. The breast is blue, the edges of the wings are violet, and the rest of the plumage, except for the black ventral feathers, shows the usual spotted design. The central tail feathers are elongated and pointed. This guinea fowl is slender and has longer legs than the helmeted guinea fowl (Ayorinde, 2004). Their advertising call is similar to that of the crested and the plumed guinea fowl, but is a much higher- pitched “keek-keek-keek,” also given in series. Vulturine guinea fowls also feed on a range of plant and animal items and will sometimes perch in bushes to feed on fruits. Unlike all other guinea fowls, this species appears to survive without readily- available drinking water (Martinez, 1994).

#### 2.1.5 Production Systems of Guinea Fowls

According to Ikani and Dafwang (2004), there are three systems of guinea fowl production. This include; the extensive system, the semi intensive system and the Intensive system.



#### 2.1.5.1 Extensive (free range) system

This system of guinea fowl management is the most common in Nigeria, and suitable for those farmers with abundant land and fields of pasture. In this management system, the birds are not confined and thus are free to fend for their own feed and roost, as well as ridding the field of insect pests and weed fruits. Because the birds find their own food and shelter, the management is almost at no cost to the farmer (Maganga and Haule, 1994). However, the free-range management system cannot be practiced on an intensive commercial scale as the birds could easily revert to the feral state and might not be easily caught when needed (Maganga and Haule, 1994). Also, on free range the birds are exposed to extremes of climatic conditions, which often results in heavy losses through predatory beast, parasites and infectious diseases. This system of production is therefore, not recommended for intensive commercial scale, but for the small-scale back yard production (Ikani and Dafwang, 2004). In most developing countries, guinea fowl farming is based mainly on the free range-system (scavenging system), which is characterized by low productivity (Mallia, 1999).

#### 2.1.5.2 Semi intensive system

This system requires a permanent housing with attached fenced runs or pasture. They should be in the minimum, two pasture areas. The birds should have access to each plot in turn, while the other pasture is rested, and the number of birds raised depends on the amount of land available. This method is particularly suitable where land is limited and in small holder farms. Under the semi intensive system, disease conditions could be common and therefore requires close monitoring and control. At the present levels of guinea fowl production in Nigeria, this system seems suitable and therefore recommended (Ikani and Dafwang, 2004).

### 2.1.5.3 Intensive system

Intensive rearing of guinea fowl, like is done for chickens, can be on the deep litter or in battery cages. Keeping layers in cages resulted in higher egg number than on the deep litter (Ayorinde, 2004). Ayorinde and Ayeni (1987) reported that raising guinea fowls in battery cages is also more advantageous than on the deep litter in that it resulted in higher body weight gain, feed efficiency, lower mortality and higher weight. The possibility of integrating guinea fowl into fish production system (Ita *et al.*, 1986) has also been exploited by keeping the guinea hens in battery cages in a poultry house constructed over a fishpond. The faecal dropping of the guinea fowls was used to fertilize the pond that was stocked with different fish species. This resulted in faster growth and better cropping of the fishes. Although adult guinea fowls are strong and hardy, young guinea fowls are frightful, active, timid and nervous and thus require special care and handling. If care is not taken, keet mortality, especially during the first two weeks following hatching, can be high (Okaeme *et al.*, 1986; Ayorinde, 1988). Adequate warmth and ventilation are required and disturbances and visits should be minimal. The corners of the brooder room should be rounded off to avoid piling up and boards can be used as guards during the first two weeks to ensure that keets receive adequate warmth and do not wander away from the feeding and drinking points. A starting temperature of 35 to 40°C that is decreased by about 2°C every week was found adequate. Heat can be supplied using electric bulbs, coal pots or kerosene stoves and lanterns(Okaeme *et al.*, 1986; Ayorinde, 1988).

Stocking rates of 0.05 – 0.06 m<sup>2</sup> on the floor and 0.048 m<sup>2</sup> in battery brooders per bird were considered adequate to 12 weeks of age (Okaeme *et al.*, 1986; Ayorinde and Ayeni, 1987; Ayorinde, 1988). This is at a rate of 10 to 20 birds per m<sup>2</sup> on the deep litter and 20 to 25 per m<sup>2</sup>

in battery cages. However, having more than 16 birds per m<sup>2</sup> was observed to result in poor growth rate, poor feed efficiency, high losses from birds struggling for feeding or drinking spaces or by suffocation through piling up especially at night or when disturbed (Ayorinde, 2004).

Older birds can be stocked at the rate of 6 - 8 birds per m<sup>2</sup>. The day old keet is covered with the fluffy hair-like feathers but in two to three weeks the primary and secondary feathers emerge and the birds can fly short distances (Ayorinde, 1999). Therefore after about four weeks of age, perches could be provided at the rate of 1.5-3.0 cm per young bird and 10-15 cm per adult bird (Ayorinde, 1988). Flightiness is preventable by monthly clipping of the flight feathers. However, a more permanent solution through pinioning was reported by Ayorinde (1987). This was done by cutting off the last portion of one wing at the last joint usually on one wing before two weeks of age for birds raised intensively only.

#### 2.1.6 Sexing of Guinea Fowls

It is difficult to differentiate the male guinea fowl from the female one. This is because there is so little difference in their appearance that many farmers find it not easy to distinguish them particularly in the young ones. Farmers who are inexperienced in keeping these birds may unknowingly keep all males or all females as breeding stock (Awotwi, 1987). When keets are gently handled from about the fourth week of age, it is possible to identify a rudimentary phallus in the males, which distinguishes them from the females. The phallus becomes fully developed and protrudes when a slight pressure is applied on the cloaca when the bird is about three months of age. At this age, the female exhibits a labia-like structure in the cloaca or there may be no structure at all (Teye and Gyawu, 2002). However, sex may easily be distinguished between the

cocks from the hens by the difference in the cry of the birds and by the larger helmet and wattles and the coarser head of the males. The cry of the hen sounds like “buckwheat, buckwheat” or “put rock, put-rock” which is quite different from the one-syllable shriek of the cock (Bell and Smith, 2003). When frightened, both the cock and the hen make one-syllable cries, but at no time does the cock’s cry sound like “buckwheat, buckwheat”. Sexing the birds by this way is possible when the birds are about 8 weeks of age. In keets (between 12-15 weeks of age) the wattles have thicker edges than do those of the females (Ikani and Dafwang, 2004).

#### 2.1.7 Breeding of Guinea Fowls

Breeding is indiscriminate; hence in-breeding is common. The mating ratio is 1 male to 5-10 females. However, a mating ratio of 1 male to 5 females appears to give optimal fertility (Moreki, 2009). However, for the small scale farmer who keeps the birds on the range, the practice of keeping fewer number of cocks in a flock is better (Ayorinde, 1987). This is because the cock often prepares the nests for a group of guinea hens that flock with him. This is why it is common to find 20 to 30 eggs in a single nest during the egg producing season in the wild (Maganga and Haule, 1994). The nests are usually located in well hidden places, making it difficult for the farmer to locate the nests when many males are kept. Such eggs also may be of poor fertility due to the monogamous tendency of the males (Ayorinde, 1987). Once the egg nests are located, farmers are advised to leave at least three newly marked or dummy eggs in the nests during each collection to encourage the guinea fowl hen to continue using the same nest during the breeding next season (Ikani and Dafwang, 2004).

In the wild, reproductive pairs are established during the rains, the pairs and their offsprings

merge together with others to form larger groups at the end of the breeding season. Thus in improved husbandry programme, efforts should be made to ensure continuous breeding and elimination of permanent pair bonds between reproductive adults (Ikani and Dafwang, 2004).

### 2.1.8 Egg Production

Guinea fowls come into lay at about 25 to 28 weeks of age and will continue to lay for about 8 months producing 150 to 160 eggs during the first laying period (Ayorinde and Okaeme, 1984). Bell and Smith (2010) in Australia reported that guinea fowl hens start laying in spring (with increasing daylight) and continue laying for about nine months. The egg-laying period can be extended and early fertility improved by using artificial lighting. In Ghana, Konlan *et al.* (2011) argued that guinea fowl hens (pearl) are capable of laying fertile eggs throughout the year when given adequate supplementary feeds with the provision of water *ad libitum*. The egg production efficiency, however depends on breeding stock and management (Oke *et al.*, 2003). Egg collection should be done daily but do not disturb the hens while they are laying. Guinea fowl eggs are smaller than chicken eggs. They weigh 35 to 40 grammes as compared to 45 to 55 grammes for chicken eggs. The eggs collected should be stored in cool dry place (Ikani and Dafwang, 2004).

#### 2.1.8.1 Egg Incubation

The two different methods of incubating guinea fowl eggs are;

##### 2.1.8.1.1 Natural incubation

The incubation period of guinea fowl egg is between 26 to 28 days. The natural incubation method is commonly used by farmers with small flocks (Avornyo *et al.*, 2007). The chicken hens

are usually used because they are more adaptable than guinea fowl hens which are too wild to be set anywhere except in the nests where they have become broody (Bell and Smith, 2003). From 12 to 15 eggs may be set under a guinea fowl hen, while 20 to 28 eggs can be set under a large chicken hen (Ikani and Dafwang, 2004).

#### 2.1.8.1.2 Artificial incubation

The artificial method of incubation is by the use of an incubator. The incubators which are of different types have in-built devices for the production of controlled heating at recommended temperature, controlled humidity level and egg turning ability (Bell and Smith, 2003). Recommended temperature and humidity of the air within incubators are about the same for both guinea fowl and turkey eggs. Temperature level of 38°C, with 58% humidity for the first 3 weeks are recommended (Ayorinde, 1989). But the temperature should be lowered to 36°C while the humidity should be raised to 75% for the last week in the incubator. Each egg should be turned at least 4 to 5 times daily for the first 24 days of incubation (Nwagu, 1997). Kerosene incubators are recommended for rural farmers who keep small flock and have no access to electricity (Ikani and Dafwang, 2004). The Nigeria Veterinary Research Institute (NVRI)-made kerosene incubators is good enough for Nigerian guinea fowl small-holder egg hatching. In automated incubators egg turning is automated while in manual incubators eggs are turned by hand. For this reason, hatchability of manual incubators is usually low (Moreki, 2009).

#### 2.1.9 Keets Brooding

Brooding of keets could be done by the natural method or by artificial brooding.

#### 2.1.9.1 Natural brooding of keets

Guinea fowl hens are not highly reputed for the care of their young ones. It will be necessary to separate the newly hatched keets from their mothers. Guinea fowl hens are likely to take their keets through wet grass and lead them too far from home (Ayorinde, 1988). The common practice, is to give out newly hatched keets to a broody chicken hen to raise. A large chicken hen will brood as many as 25 keets (Ayorinde, 1990). For the first 2-3 days the hen and keets should be confined to an enclosure, after that time they should be allowed to range. However, shelter should be provided at night to keep out predatory animals (Maganga and Haule, 1998). Keets raised by natural method will usually leave the brooders house from the age of 6 to 8 weeks and will begin roosting at night in a nearby trees in the open air (Ayorinde, 1990). But if they have been accustomed to going into the house at night in company of the chicken mother hen they are so trained and will not be so difficult to catch when they are wanted for the market (Ikani and Dafwang, 2004).

#### 2.1.9.2 Artificial brooding

The newly hatched keets may be raised with the same kinds of brooders and brooden houses as are used for chickens or turkeys (Ayorinde, 1988). The recommended brooding methods and temperatures are similar to those used for chickens. Artificial brooding using electric bulbs can be started at a temperature range of 34-40°C for the first 3 weeks (Okaeme *et al*, 1986; Ayorinde, 1988). This should be reduced to 30-35°C between 4 and 6 weeks and 28-32°C as from 7 to 8 weeks (Ayorinde, 1988). Thereafter, heating can be discontinued except during the cold seasons. These temperatures can be attained using 12 x 60 watt bulbs per 50m floor space or 18 x 40 watts bulbs at about 15cm above the floor (Ayorinde, 1988). Kerosene lamps could also be used

as an alternative and readily available source of producing warmth for young guinea fowls. The brooder house should be constructed to provide both warmth and adequate ventilation for the keets and located where there will be least disturbance, which could cause losses due to pilling up as a result of frights. Overcrowding should be avoided and any form of disturbances e.g. noise, too frequent visits and sight of other animals. Corner guards are useful to prevent pilling up while pinioning of wings helps to reduce flightiness (Ikani and Dafwang, 2004).

#### 2.1.10 Housing and Equipment

Commercially, guinea fowls are kept confined in suitably adapted buildings on a 75mm litter depth of wood shavings. Adequate ventilation is important because the droppings are much drier than those of other poultry and this leads to a dustier atmosphere and therefore to an increased risk of respiratory diseases. Over stocking should therefore be avoided (Ikani and Dafwang, 2004). The floor of rearing pen should be cemented and slightly slopped to facilitate easy cleaning and washing. “Dwarf walls” (1m high) made of block or dried brick or mud are adequate (Ayorinde and Ayeni, 1987). On top of the wall should be a wire mesh of 2.5m high supported by wooden frames. Ventilation holes should be made into the walls but with movable cover of plank to regulate heat. Roof could be made of asbestos to ensure cooling (Ikani and Dafwang, 2004).

##### 2.1.10.1 Space requirement and stocking density

Floor space requirement of about 0.06m should be allowed per bird from day old till maturity. Stocking density is at the rate of 100 birds per 55-65m. A stocking rate of more than 16 birds per meter is overcrowding and can result to heavy losses (Ayorinde, 1988). Up to 50% of the entire



flock could be lost through such over stocking. Over stocked birds also look unthrifty and do not normally grow well. It is also advisable not to under stock, as the birds, especially when young tend to wander far away from sources of heat, food and water, which often cause death due to starvation (Ayorinde, 2004). In order to ensure optimum performance, rearing size should not exceed a thousand birds. Equipment for rearing of guinea fowls are the same with that of chickens which includes drinkers, feeders, and nest boxes, while lighting devices are optional (Ikani and Dafwang, 2004).

#### 2.1.10.2 Feeder and drinker requirement

In intensive method of production, few hours (2-3h) before the newly hatched keets are received on the farm, feed should be placed on pieces of paper or flat trays, while drinkers should be placed around to get the birds familiar with them (Maganga and Haule, 1994). The drinkers should be filled with clean cool water, and positioned about 1m from the wall but away from sources of heat. Coloured feeders and drinkers are preferred. Red colour is favoured because it attracts the keets (Ikani and Dafwang, 2004). Drinking spaces of 1cm, 1.5cm - 3.0cm and 3.0-5cm per bird should be allowed for the first 4 weeks, 5-12 weeks and 12-16 weeks old, respectively (Ikani and Dafwang, 2004).

#### 2.1.11 Health Care and Management of Guinea fowls

The period or times guinea fowls experience the highest mortality throughout their lives is during the keet stage (0 to 8 weeks old). During this stage, guinea fowls are very fragile, and lose their lives at the least mistake. Maganga and Haule (1994) reported that a fairly high mortality can be expected with the keets up to the age of six weeks. According to Avornyo *et al.* (2007), the

causes of mortality include ill health, cold, rain beating keets, accidents and predation. The losses can be prevented by farmers improving upon their management practices. Young keets should be housed for a minimum of four to eight weeks. While in confinement, the keets need to be provided with the required space, warmth, lighting, feed, medication and water (Avornyo *et al.*, 2007).

The major helminth parasites that have been reported infecting guinea fowls in Nigeria are such like *Heterakis* spp and *Ascaridia galli* while *Eimeria* spp are the most important gastro intestinal protozoan parasites (Matur *et al.*, 2010). It is also known that *A. galli*, *Heterakis* spp and *Eimeria* spp are responsible for deaths especially among the young ones (Luka and Ndams, 2007). The practice in most farms, with considerable success had been to treat guinea fowls with the same drugs as those recommended for the treatment of other poultry, particularly chickens (Pamet *al.*, 2006). The study of Bonkougou (2005) in Burkina Faso reported that guinea fowl are more tolerant to common viral and bacterial diseases that occur in poultry but are intolerant to internal and external parasites because of their scavenging behaviour under semi-intensive production systems. Teye and Gyawu (2001) in Ghana observed that although the birds generally exhibited considerable tolerance to common avian diseases, leg paralysis and yolk sack infection commonly occurred.

Some commonly reported diseases of guinea fowls in Nigeria and Africa at large are Newcastle disease, coccidiosis, helminthiasis, colisepticaemia, salmonellosis, trauma, pediculosis and hardware disease (Saidue *et al.*, 2004; Moreki *et al.*, 2011; Hassan *et al.*, 2014). As guinea fowl is mainly in the hands of smallholder farmers, disease and parasite control involves both the use of

modern medicines and ethnoveterinary medicine with the latter predominating (Gueye, 1999). As a result, keet mortalities are high, indicating that technical support is critical (Moreki *et al.*, 2011). In guinea fowl, like other domestic birds, prevention is obviously better than cure and you can avoid diseases a great deal by good sanitary conditions (Ikani and Dafwang, 2004).

### 2.2.1 *Ascaridia galli*

Parasitism is one of the major problems which inflict heavy economic losses to the poultry in the form of retarded growth, reduced weight gain, decreased egg production, diarrhoea, and obstruction of the intestines, poor feathers, replacement birds that take long to reach maturity, morbidity and mortality. Stress from parasites could affect the blood picture and cause anorexia (Shah *et al.*, 1999; Dube *et al.*, 2010). *Ascaridia galli* is a parasitic roundworm that belongs to the superfamily Ascaroidea within the Class Nematoda and it occurs worldwide in galliform birds of all ages. The adult worms live in the lumen of the intestine, but are occasionally also found in the crop, gizzard and rarely in the oviduct or body cavity (Ramadan and Znada, 1991). It is one of the largest nematodes of the small intestine of birds. It may cause reduction in growth rate, weight loss, sometimes serious illness, pathological lesions and economical losses in native birds such as hens, turkeys, guinea fowls, geese and some other birds (Ramadan and Znada, 1991). It can also lead to damage to the intestinal mucosa leading to blood loss and secondary infection and occasionally, obstruction of small intestine of poultry due to high worm burden may occur (Soulsby, 1982). A Danish study reported an estimated prevalence of 64% in free-range and organic poultry flocks and 42% flocks on deep-litter systems (Permin *et al.*, 1999). The most important clinical sign of *A. galli* infections is loss of body weight, which increases parallel to worm load (Reid and Carmon, 1958). Increased feed intake (Gauly *et al.*, 2007), blood loss,

reduced body weight gain, and increased mortality may also occur (Ikeme, 1971). Economic losses and welfare issues that result from severe *A. galli* infections are important problems in laying flocks, especially in birds kept in free-range and organic farming systems (Ruff, 1999; Martin-Pacho *et al.*, 2005).

It has also been reported that parasitic infection or their concurrent infections result in immunosuppression, especially in response to vaccines against some poultry diseases (Nnadi and George, 2010). Helminth parasites of poultry are commonly divided into three main groups; nematodes, cestodes and trematodes. Nematodes constitute the most important group of helminth parasites of poultry both in number of species and the extent of damage they cause; the main genera include *Capillaria*, *Heterakis*, and *Ascaridia*. The cestodes of significant importance are of the two genera *Railleitia* and *Hymenolepis* (Matur *et al.*, 2010). In the commercial table egg production systems the most commonly occurring helminth species are *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria spp* (Roy, 2002). *Ascaridia galli* has been incriminated as the most common and most important parasite of poultry (Pam, *et al.*, 2006; Luka and Ndams, 2007).

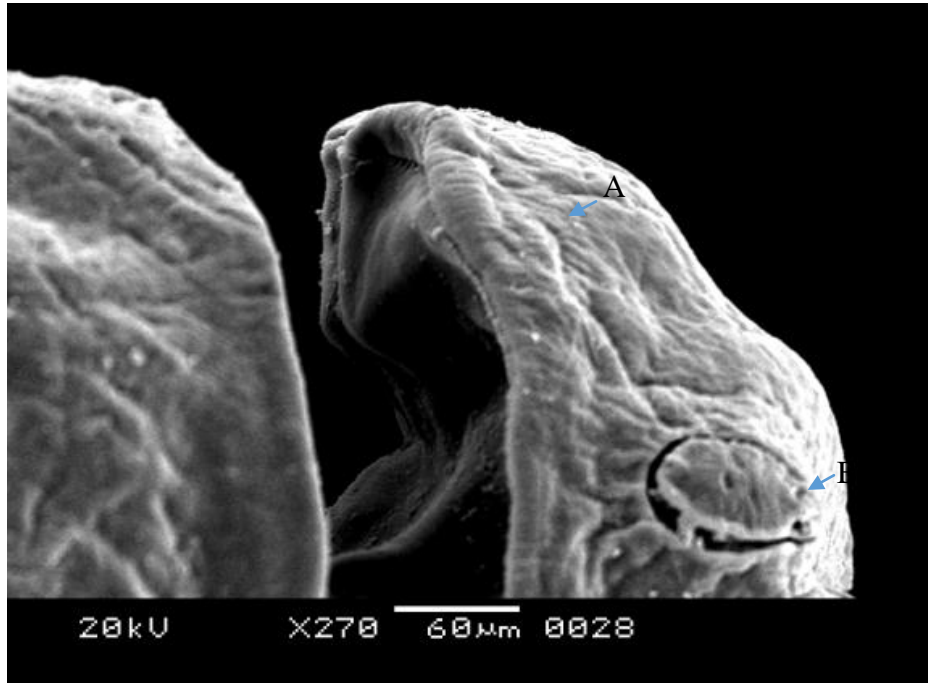
#### 2.2.1.1 Description of *Ascaridia galli*

*Ascaridia galli* is the largest nematode in birds. The body is semitransparent, creamy-white and cylindrical (Hassanain *et al.*, 2009). The anterior end is characterized by a prominent mouth, which is surrounded by three large tri-lobed lips. The edges of the lips bear teeth-like denticles (Ashour, 1994). The body is entirely covered with a thick proteinaceous structure called cuticle. The cuticle is striated transversely throughout the length of the body and cuticular alae are poorly

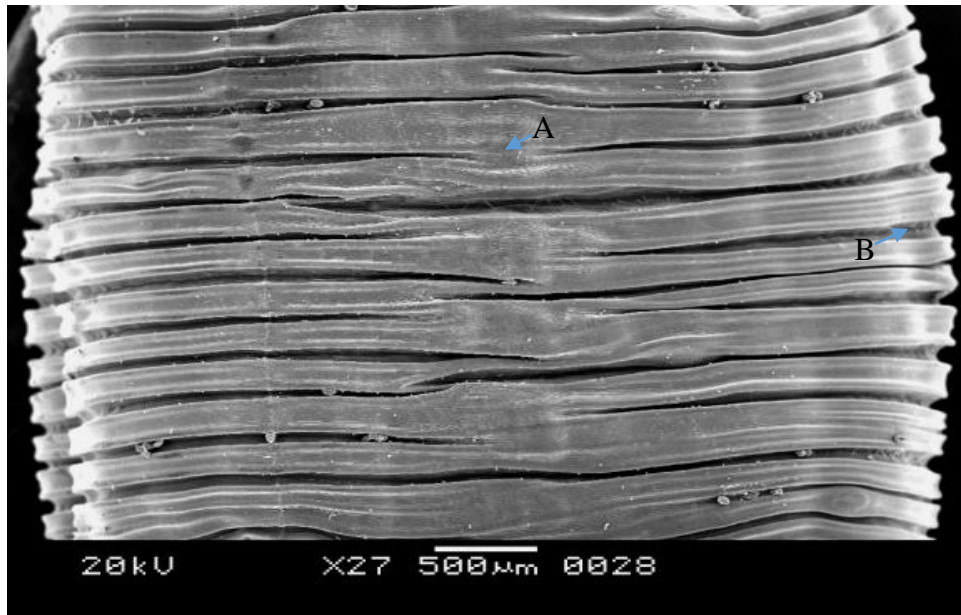
developed (Ramadan and Znada, 1992). Two conspicuous papillae are situated on the dorsal lip and one on each of the subventral lips (Lalchhandama *et. al.*, 2009). These papillae are the sensory organs of the nematode. *A. galli* is diecious with distinct sexual dimorphism. Females are considerably longer and more robust, with vulva opening at the middle portion (approximately midway from anterior and posterior ends) of the body and anus at the posterior end of the body. There are also ten pairs of caudal papillae towards the tail region of the body, and they are arranged linearly in well-defined groups such as precloacal (3 pairs), cloacal (1 pair), post-cloacal (1 pair) and subterminal (3 pairs) papillae (Lalchhandama, 2010).

#### 2.2.1.2 Life cycle of *Ascaridia galli*

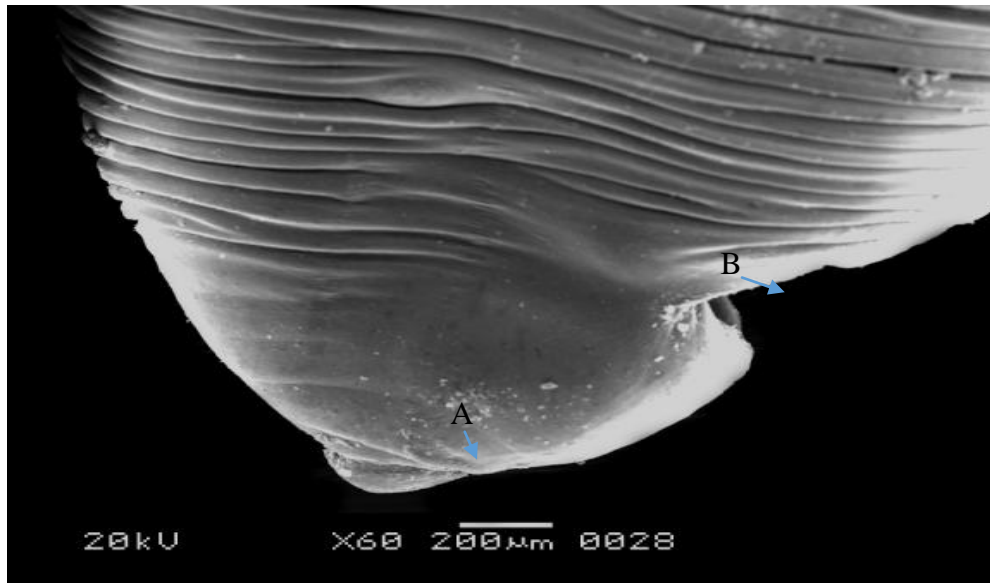
*Ascaridia galli* has a simple, direct lifecycle. Infective eggs are ingested and hatch in the proventriculus. The resulting larvae live in the duodenum for a short time before penetrating the intestinal mucosa. This migration through the intestinal lining can result in anemia and enteritis (Gauly *et al.*, 2007). [Larvae](#) do not hatch but [moult](#) inside the eggs until they reach the L3 stage. This can take about two weeks but the period depends on other factors such as the weather condition. The life cycle is completed when the infective eggs are ingested by new hosts through contaminated water or feed. The eggs containing the L3-larvae are mechanically transported to the [duodenum](#). The infective eggs are ingested by a chicken where it reaches the [proventriculus](#) and hatches. Temperature, [carbon dioxide](#) levels and [pH](#) are thought to be triggering factors that signal the larva to hatch from its egg (Ramadan and Znada, 1992). The larva then burrows into the [mucosal](#) lining of the small intestine where it undergoes two additional [moult](#)s. It is this phase of their life cycle where these worms cause the most damage to their host.



**Plate I:** Electron micrograph of an adult *Ascaridia galli* towards the anterior end. The mouth, which is entirely composed of lid-like structures called lips (arrow A) and a distinct eye-like oval structure that is the papilla (arrow B).  
Source: Lalchhandama (2010)



**Plate II:Electron micrograph of an adult *Ascaridia galli* towards the middle portion of the body. The cuticle (arrow A) is finely corrugated giving rise to a series of unique transverse striations called annulations (arrow B).  
Source: Lalchhandama (2010)**



**Plate III:** Electron micrograph of adult female *Ascaridia galli* towards the posterior portion of the body. The female has a characteristic straight and blunt tail end (arrow A); an anal opening can be seen at the right hand side (arrow B).

Source: Lalchhandama (2010)

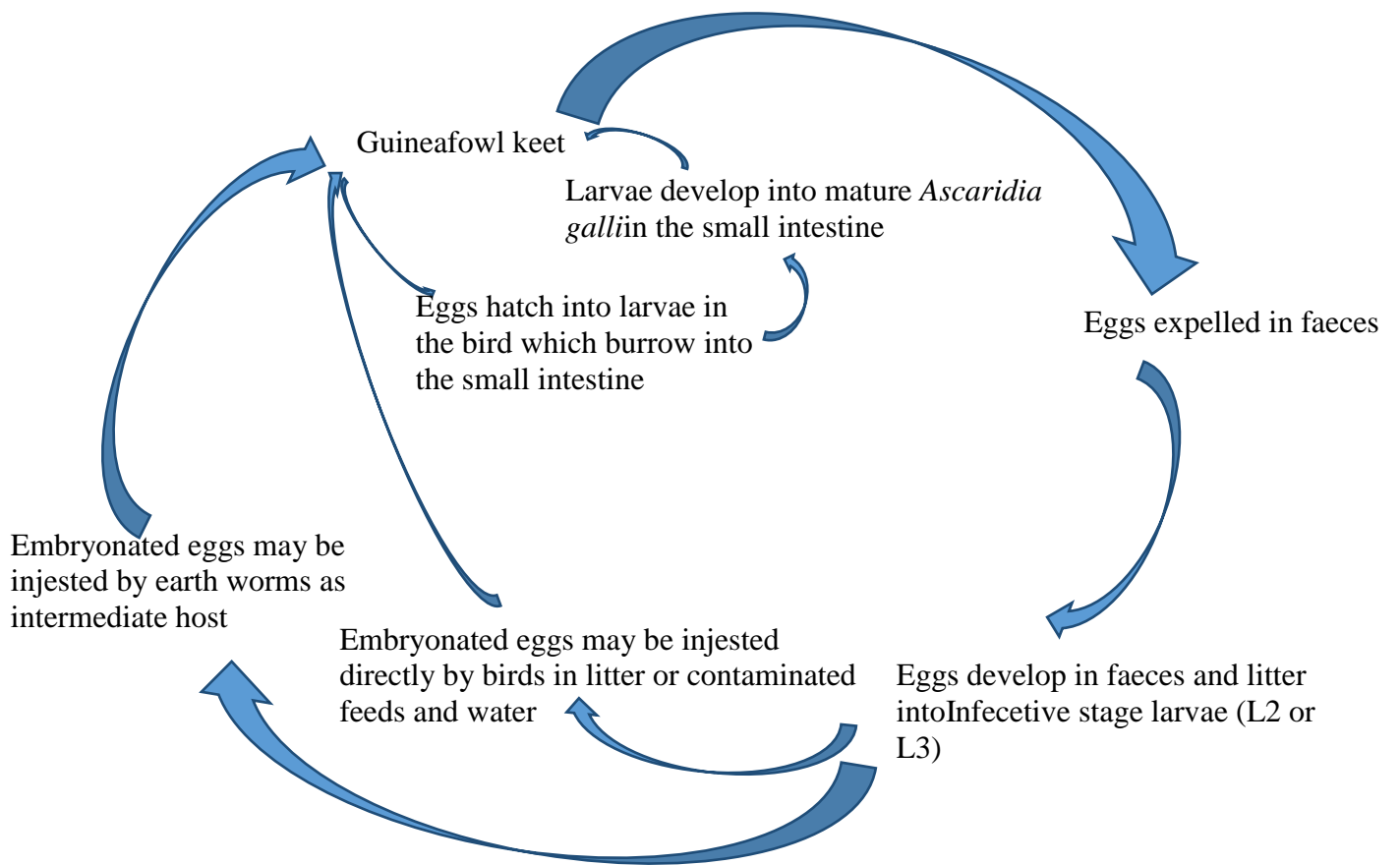




**Plate IV:**Electron micrograph of adult male *Ascaridia galli* towards the posterior end of the body.

It is characterized by an elaborate, finely pointed tail with two particularly distinct ring-like structures; towards the extreme terminal is the anus (arrow B) and anterior to it is the precloacal sucker (arrow A), which serves as the holdfast during copulation.

Source: Lalchhandama (2010)



**Figure 2.1:**Life circle of *Ascaridia galli*(Gauly *et al.*, 2007).

They then re-enter the small intestine and develop into adults where they live their lives out feeding on [gut](#) content and producing a vast amount of eggs that would then be excreted by a host and free to continue their life cycle. If the animal is able to mount an [immune response](#) to the larvae, i.e. from pre-exposure, the larvae do not develop into adults but hide in the [mucosa](#) of the small intestine. This is common for infection of older birds. Transport hosts such as [earthworms](#) are thought to play a role in transmission of *A. galli* and hence, [free range](#) birds tend to have a higher risk of infection (Anderson, 2000).

#### 2.2.1.3 Pathogenicity of *Ascaridia galli* infestation

The nematode infects fowl of all ages, but the greatest degree of damage is often found in young birds under 12 weeks of age. Heavy infection is the major cause of weight depression and reduced egg production in [poultry husbandry](#). In severe infections, intestinal blockage can occur as well as unthriftiness, drooping of the wings and [emaciation](#). It also causes loss of [blood](#), reduced [blood sugar](#) content, increased [urates](#), shrunken [thymus glands](#), retarded growth and greatly increased [mortality](#). In heavy infections, adult worms may move up the [oviduct](#) and be found in hens' eggs, and sometimes they are also found in the birds' [feces](#) (Jacobs *et al.*, 2003). Infections with *A. galli* can cause growth depressions and lower nutrient utilization, which may be related to damages in the intestinal mucosa (Ramadan and Znada, 1991; Gauly *et al.*, 2005) that adversely affects the absorption of nutrients in the intestine, leading to blood loss, secondary infections and behavioural changes (Soulsby, 1982; Gauly *et al.*, 2007). It has been shown that these effects are more severe when animals are infected not only by *A. galli* but also by other pathogens (Chadfield *et al.*, 2001; Dahl *et al.*, 2002). Severity of the intestinal lesions may depend on the number of worms established in the intestine (Ikeme, 1971).

#### 2.2.1.4 Treatment of *Ascaridia galli* infestation

[Piperazine](#) is one of the drugs of choice. Continuous medication in feed with [hygromycin B](#) is also widely employed. Piperazine may be administered to chickens in the feed (0.2-0.4%) or water (0.1-0.2%), or as a single treatment (50–100 mg/bird). However, piperazine is quite ineffective for young chickens, while tetramisole is 89-100% effective for chicken of different ages (Pavlíček and Dyková, 1976). Drugs such as [albendazole](#) and [levamisole](#) are also highly effective (Sharma *et al.*, 1989). [Fenbendazole](#) is also very effective, 99.2-100% and 69.0-89.6% effective at administration doses of 60.6 [ppm](#) and 30.3 ppm (Sander and Schwartz, 1994). [Ivermectin](#) was also demonstrated to be 90 and 95% effective against immature and adult worms, respectively (Sharma *et al.*, 1990).

### 2.3 Haematological Parameters

#### 2.3.1 Packed cell volume

Packed cell volume (PCV) is an important hematologic assay because it provides an easy and objective way of estimating the number of erythrocytes in the sample. It is also essential for the calculation of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). In avian species, PCV is best estimated using the microhematocrit method. The use of plain microcapillary tubes is preferable, since the same tube can be subsequently used to estimate fibrinogen (Pendl, 2001).

#### 2.3.2 Haemoglobin estimation

In avian species, estimation of haemoglobin is hampered by the presence of nuclei in the erythrocytes. Haemoglobin estimation relies on the colorimetric measurement of haemoglobin

released after the lysing of the erythrocytes(Fudge, 1997). Haemoglobin can be estimated using automatic methods or manual methods.Commercial laboratories that estimate hemoglobin using an automatic hematology analyzer have to take into consideration the photometric interference of the free nuclei after lysing of the erythrocyte(Quinto *et al.*,2006).

In the manual method, it is essential to remove the nuclei from the preparation because its presence could yield unreliable results. The nuclei can be sedimented by low-speed centrifugation, but because some haemoglobin remains attached to the nuclei, colorimetric readings are commonly low(Fudge, 1997). This can be overcome by estimating hemoglobin as cyanmethemoglobin using alkaline Drabkin's cyanide-ferricyanide solution or as oxyhaemoglobin using ammonia solution(Campbell, 1995). In both cases, the estimation is carried out using a spectrophotometer at the absorbance reading of 540 nm. A calibration graph should be made using commercially available hemoglobin standards to express hemoglobin as oxyhaemoglobin. Conversely, haemoglobin can be estimated directly as oxyhaemoglobin using a commercially available haemoglobinometer (Gulland and Hawkey, 1990).

### 2.3.3 Total white blood cell count

The total white blood cell count (WBC) is one of the most important hematology assays in the assessment of health and disease in an individual(Bain and Bates, 2001). The WBC also is useful because it is used together with the differential white cell count to calculate the absolute number of each white blood cell within a blood sample (Pendl, 2001). Avian blood differs in cells' characteristics from their mammalian counterpart (Smith *et al.*, 2000). Several factors including physiological (Alodan and Mashaly, 1999) and environmental conditions (Vecerek *et al.*, 2002;

Graczyk *et al.*, 2003; Oladele, 2009), diet contents (Odunsi *et al.*, 1999; Kurtoglu *et al.*, 2005), water and feed restriction (Galip, 1999; Al-Rawashdeh *et al.*, 2000; Iheukwumere and Herbert, 2003), fasting (Lamošová *et al.*, 2004), age (Forlan *et al.*, 1999; Naziefy-Habibabadi, 1997; Seiser *et al.*, 2000), administration of drugs (Khan *et al.*, 1994; Zaman *et al.*, 1995), anti-aflatoxin premixes (Oguz *et al.*, 2000) and continuous supplementations of vitamin E (Tras *et al.*, 2000) affect the blood profiles of healthy birds. Uko and Ataja (1996) reported that the normal haematological values of male guinea fowl in North West Nigeria were 35.96, 10.68, 3.45, 104.23, 30.96, 29.70, for PCV, Hb, RBC, MCV, MCH, and MCHC respectively, and for the female they were 33.04, 10.51, 3.40, 98.35, 30.91, and 31.45 for PCV, Hb, RBC, MCV, MCH, and MCHC respectively. Similarly, in Zaria, Nigeria, the mean PCV values for guinea fowls during the harmattan, hot and rainy seasons were  $31.41 \pm 0.80\%$ ,  $24.80 \pm 1.09\%$  and  $32.30 \pm 0.31\%$ , respectively, while the mean Hb values for guinea fowls during the harmattan, hot and dry seasons were  $10.55 \pm 0.45 \text{ g/dl}$ ,  $8.33 \pm 0.43 \text{ g/dl}$  and  $12.80 \pm 1.12 \text{ g/dl}$ , respectively (Oladele *et al.*, 2005).

## **2.4 Serum Biochemical Parameters**

### **2.4.1 Aspartate aminotransferase**

Aspartate aminotransferase (AST) also called serum glutamic oxaloacetic transaminase is similar to ALT in that it is another enzyme associated with liver parenchymal cells (Xing-Jiu *et al.*, 2006). It is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle, so is not specific to the liver. AST is present in both the cytoplasm and mitochondria of hepatocytes (and many other cells) and will elevate in states of altered membrane permeability (Kirsch *et al.*, 1984). In such cases, levels are expected to be less than in states of

frank necrosis, when both cytoplasmic and mitochondrial enzymes are released (Almo *et al.*, 1994). The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. Elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker (Nyblom *et al.*, 2004).

#### 2.4.2 Alanine aminotransferase

Alanine aminotransferase (ALT) is considered to be liver-specific in small animals. This enzyme is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. ALT is a cytoplasmic enzyme, and is considered to be liver-specific in dogs, primates and some other small animal species. There is little hepatic ALT activity in large animals (Nyblom *et al.*, 2006). Elevation of serum levels of both AST and ALT can occur with states of altered hepatocellular membrane permeability. Because ALT is located only in the cytoplasm, serum levels tend to be relatively higher than AST, as a result of membrane leakage from the hepatocyte. Mitochondrial enzymes are less likely to be released with most of the conditions which result in increased membrane permeability (Nyblom *et al.*, 2006). Uko and Ataja (1996) also found that the blood chemistry values for male guinea fowl in Nigeria were 115.6, 55.3, 4.4, 1.9 and 2.5 for Cholesterol, AP, total protein (Tp), Albumin (ALB), and Globulin respectively. Olayemi *et al.* (2002) reported that the ranges of AST and ALT were 155-171.67 and 9.17-12.50 i.u/l respectively for adult guinea fowls.

### 2.4.3 Alkaline phosphatase

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma rise with large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodelled) and elderly patients with Paget's disease. In the third trimester of pregnancy, ALP is about two to three times higher (Nyblom *et al.*, 2004).

## 2.5 *Xylopi aethiopica*

The aromatic plant *Xylopi aethiopica* Dunal (Annonaceae), commonly known as “African pepper”, “Ethiopia or Negro pepper” has been used in Europe, Asia and Africa as pepper substitute and spice in local cooking. In Nigeria, the common local names used in different languages to refer to this plant are: *Kimba* in Hausa, *Eeru* in Yoruba and *Udain* Igbo (Abolajiet *al.*, 2007). Various parts of the plant have been traditionally employed in different therapeutic preparations. The mature fruits of green color take a brown-black coloration after drying and are used as spices (Fall *et al.*, 2003; Ogunkunle and Ladejobi, 2006).

### 2.5.1 Chemical composition *Xylopi aethiopica*

Chemical components of *Xylopi aethiopica* have been helpful in the prevention and treatment of cancerous tumors (Del-Rio *et al.*, 1997). *Xylopi aethiopica* fruits contain alkaloids, flavonoids, terpenoids, fixed oil and volatile aromatic oil. Key constituents are diterpenic and xylopic acids. *Xylopi aethiopica* oil contains carbohydrates and glycosides (Iwu, 1993; Shanmugam *et al.*, 2008).



### 2.5.2 Botanical background of *Xylopia aethiopica*

*Xylopia aethiopica* (Dunal) A. Rich is a slim, tall tree of about 60–70 cm in diameter that can reach up to 15–30 m tall, with a straight stem and a slightly stripped or smooth bark. The fruits are rather small and look like twisted bean-pods. When dry, the fruit turn dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick. The contours of the fruits are visible from outside. Each pod contains 5 to 8 kidney-shaped fruits of approximately 5 mm in length. The hull is aromatic, but not the fruits (Tairu *et al.*, 1999).

### 2.5.3 Pharmacological properties and chemical composition of *Xylopia aethiopica*

The plant contains anonaceine, an alkaloid, and rutin, a volatile aromatic oil and a fixed oil. A pharmacological investigation of fruit extract against skin infection has shown modest activity against Gram-positive organisms. The plant contains high amounts of copper, manganese, and zinc. Key constituents are diterpenic and xylopic acids, and these within the fruit extracts show activity as an antimicrobial against Gram-positive and Gram-negative bacteria. However, it has not been shown to be effective against *E. coli* (Iwu, 1993). Xylopic acid has also demonstrated activity against the fungus *Candida albicans*. The essential oil has been well characterized with linalool,  $\beta$ -trans-ocimene,  $\alpha$ -farnesene,  $\alpha$ -pinene,  $\beta$ -pinene, myrtenol,  $\beta$ -phellandrene, and 3-ethylphenol as the major volatile constituents (Tairu *et al.*, 1999). Researchers describe that the intense ‘pepperish note’ of the oil of the fruit largely comes from linalool and provides that characteristic aroma of the ground, dried, smoked fruits of *Xylopia aethiopica*. The essential oil yield varies from 2.0% to 4.5%. The essential oils of the stem bark (0.85%) and the leaves (0.5%) of *X. aromatica* have also been investigated. The bark oil consists mainly of pinene, trans-

pinocarveol, verbenone and myrtenol and differs significantly from that of the leaf oil (spathulenol, cryptone, beta-caryophyllene and limonene)(Tairu *et al.*,1999).

#### 2.5.4 Medicinal uses of *Xylopi aethiopica*

*Xylopi aethiopica* is common in ethno-medicine in West Africa. This is due to its preservative effect. The fruit extract has been shown to be active as antimicrobial agent against Gram-positive and Gram-negative bacteria, though it has not been shown to be effective against *Escherichia coli*. (Iwu, 1993). *Xylopi aethiopica* has anti-spirochoetal properties so that it works both as a preventive measure and in treatment of primary, secondary and tertiary stages of syphilis (Mitra, and Misra, 1967). *Xylopi aethiopica* has been used for treating rheumatism and arthritis as well as other inflammatory conditions. Numerous studies have confirmed the spice's anti-inflammatory and antipyretic properties (Sofowara, 1978). Indian researchers reported antiarthritic and anti-inflammatory actions of one of the compounds of *Xylopi aethiopica* called nimbidin (Pillai and Santhakumari, 1981).

The fruits are mainly used by traditional medicine healers and can also serve as an alternative to pepper (Agoha, 1974). Medical uses of the plants are: as acarminative, as cough remedies and as a post-partum tonic and lactation aid. Other uses include treatment for stomach ache, bronchitis, biliousness and dysentery (Agoha, 1974). It has also been reported to be used as a flavour in palm wine. The bark of tree when steeped in palmwine is given for attacks of asthma and rheumatism (Smith *et al.*, 1996). It is widely accepted that fruits and vegetables have many healthful properties.

There are considerable amount of epidemiological evidences revealing an association between those who have a diet rich in fresh fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer (Del-Rio *et al.*, 1997). The constituent of these fruits and vegetables that contribute to these protective effects are phytochemicals, vitamins and minerals (Okwu, 2003). Naturally occur ring phytochemicals like flavonoids are potentially anti-allergic, anti-carcinogenic, antiviral and antioxidant agents (Close and McArthur, 2002). Phytochemicals as antioxidants play vital roles in human health (Del-Rio *et al.*, 1997). *Xylopia aethiopica* has been found to contain some phytochemicals which exhibit a wide range of biological effects as a consequence of their antioxidant properties (Close and McArthur, 2002). Researchers in India, Europe and Japan have found that polysaccharides and limonoids found in *Xylopia aethiopica* reduce tumors and cancers (Grahamet *al.*, 2000).

Flavonoids prevents oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis (Salah *et al.*, 1995). As antioxidants, flavonoids from *Xylopia aethiopica* provide anti-inflammatory action (Okwu, 2001). Anonecaine, an alkaloids constituent of *Xylopia aethiopica*, is known to have anti-pyretic effect (Clarence, 1980). Saponins another phytochemical constituent of *Xylopia aethiopica* have wide range of biological properties; they are used to recover homeostasis, have anti-inflammatory and anti-cancer actions (Sparg *et al.*, 2004). The fruit of this plant was used against cough, stomachache, dizziness, amenorrhea, bronchitis, lumbago and neuralgia(Woode *et al.*,2012). It is also used as calmative, purgative, repulsive to pain and in the treatment of boils and skin eruptions (Tairuet *al.*, 1999).

### 2.5.5 Ecology and methods of cultivation of *Xylopia aethiopica*

*Xylopia* is native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely located in West, Central and Southern Africa. These trees are widely distributed in the humid forest zones of West Africa, especially along rivers in the drier area of the region (Tairuet. *al.*, 1999). In tropical and highlands of Africa (from Ethiopia to Ghana), both species *Xylopia aethiopica* and *X. striata* occur and both are used for local cooking. In South America, a third species is of interest, *X. aromatica* (burro pepper), which has found similar applications among Brazilian Indios. The tree prefers high rainfall areas and well-drained soils. While *Xylopia aethiopica* thrives in the forest regions, the tree can also be found in transitional zones. Loamy and sandy loamy soils are conducive for the cultivation of the plant. The plant can successfully be intercropped with other staple food items such as in the first four years. Propagation is easily accomplished by fruits. Seedlings are transplanted to the field within three to five months after sowing. The plant grows rapidly the first three years. Trees are planted eight meters apart.

In West Africa, the tree flowers twice per year, March to July, and October to December. Fruiting takes place in December to March and June to September. Harvesting time runs from February to May and again from August to October. The fruits are harvested with the inflorescence. After picking, the fruits are sun-dried for four to seven days. Fruits should not be dried on the ground, but on a protective cloth, net, screen or shelving system to minimize any microbial contamination. After drying, the fruits are removed from the inflorescence stalks. Typical fruit yields are about two to three metric tons per annum per hectare (Burkill, 1985).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Source of Guinea Fowl Keets and Housing**

One hundred day-old guinea fowl keets of the Pearl variety were obtained from the Hatchery of Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria. The keets were housed in pens that were thoroughly cleaned and disinfected at the Poultry Disease Research Unit of Veterinary Teaching Hospital, Ahmadu Bello University, Zaria (Plate I). The poultry house was of the open sided type and deep litter floor. Brooding was done with 200 watts electric bulbs for the first three weeks before commencement of the research. The birds were fed commercial poultry feed (Rebson feeds<sup>®</sup>) (chick mash of 20% C P, for the first 4 weeks of age and grower mash of 15% C P from 5 weeks of age) and water *ad-libitum*. The birds were vaccinated with Newcastle disease vaccine La Sota strain orally in drinking water, at one and three weeks of age.

#### **3.2 Source of *Xylopi aethiopica* Fruit and Preparation of Extract**

Four kilograms of dried fruits of *Xylopi aethiopica* (XA) were bought in January, 2015 from Samaru Market of Sabon Gari Local Government Area, Kaduna State, Nigeria (Plate II). The dried XA fruits were grounded and weighed, after which 1.5 kg was soaked in 7.5 litres of distilled water. This was then allowed to stand for 24 h after which it was filtered using muslin cloth. The filtrate was allowed to stand for one hour, so that the suspended particles settle at the base. The clear filtrate was gently decanted into a clean evaporating dish and placed on a water

bath at 70°C to evaporate to dryness(Sofowora, 1984). The dried extract was scraped and placed in a suitable container for further analysis.

### **3.3 Phytochemical Analyses of *XylopiA Aethiopica* aqueous Fruit Extract**

Phytochemical analyses of the *Xa* fruits were carried out in the Department of Pharmacognosy and Drug Development, A B U, Zaria, using standard protocols as outline below:

#### **3.3.1 Test for carbohydrates and sugars**

Molisch's Test: 1ml of  $\alpha$ -naphthol solution was added to 2ml of the *Xa* extract and then concentrated sulphuric acid was added through the side of the test tube. Appearance of purple or reddish violet colour at the junction of the two liquids was indicative of the presence of carbohydrates(Sofowora, 1984).

#### **3.3.2 Test for saponins**

Frothing test/Foam test: 20 ml of distilled water was added to 5 ml of the *Xa* extract and shaken in a graduated cylinder for 15 minutes lengthwise. Appearance of a 1cm layer of foam was indicative of the presence of saponins(Sofowora, 1984).

#### **3.3.3 Test for steroids**

Libermann-Burchard Test: 1gm of the *Xa* extract was dissolved in 10 ml of chloroform, and then 3ml of acetic anhydride, followed by 3ml of glacial acetic acid were added, following which the mixture was warmed and cooled under tap water, and drops of concentrated sulphuric acid were

added along the sides of the test tube. Appearance of bluish-green colour showed the presence of steroids (Sofowora, 1984).

#### 3.3.4 Test for terpenoids

Liebermann-Burchard Test: 5 ml *Xa* extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated  $H_2SO_4$  was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids (Sofowora, 1984).

#### 3.3.5 Test for cardiac glycosides

Keller-kilani test: 1 ml of *Xa* extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of  $FeCl_3$ . The mixture was then poured into another test tube containing 2ml of concentrated  $H_2SO_4$ . A brown ring at the interphase indicated the presence of cardiac glycosides (Sofowora, 1984).

#### 3.3.6 Test for alkaloids

Dragendorff's Test: 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution) was added to 1 ml of the *Xa* extract. An orange-red precipitate indicated the presence of alkaloids (Sofowora, 1984).



**PlateV:Six-week-old guinea fowl keets kept on deep litter before commencement of the experiment at Poultry Disease Research Unit of the Veterinary Teaching Hospital Ahmadu Bello University, Zaria, Nigeria.**





**Plate VI:** Dry *Xylopiya aethiopica* fruits obtained from Samaru market, Zaria, Nigeria.



**Plate VII: *Xylopiya aethiopica* tree. Source: Tairu *et al.* (1999)**

### 3.3.7 Test for tannins

Braemer's test: 1-2 drops of Ferric chloride solution was added to 1ml of the *Xa*extract. Formation of a dark blue or greenish black colour indicated the presence of tannins(Tyler *et al.*, 1981).

### 3.3.8 Test for anthraquinones

Borntragers test: About 0.5 g of the *Xa*extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicated the presence of anthraquinones(Mehta *et al.*, 2013).

### 3.3.9 Test for flavonoids

The *Xa*extract (1 ml) was taken in a test tube and 2 drops of dilute NaOH solution added. The appearance of an intense yellow colour which became colourless on addition of 2 drops of dilute  $H_2SO_4$  was indicative of the presence of flavonoids(Trease and Evans, 1983).

## **3.4 Acute Toxicity Study of *Xylopi aethiopica* aqueous whole fruit extract**

Toxicity study of the aqueous *Xylopi aethiopica* whole fruits extract was carried out in Toxicology Laboratory of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The  $LD_{50}$  of the *Xa*fruit extract was determined using Lorke's method (Lorke, 1983) with modifications. Briefly, the test was carried out in two phases. Phase 1: Nine rats were divided into three groups of three rats each. The three groups were administered orally with graded doses (10, 100 and 1,000 mg/kg) of the

*Xa* extract respectively. Phase 2: Another nine rats were divided into three groups of three rats per group, which received graded doses (1,600, 2,900 and 5,000 mg/kg) of the *Xa* extract respectively. The number of deaths in each group within 24 h was recorded and the final LD<sub>50</sub> values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

### **3.5 Source of infective eggs of *Ascaridia galli***

Infective eggs of *A. galli* were obtained from live adult females of *A. galli* collected from the small intestines of birds slaughtered at Sabon Gari live bird market, Zaria, Kaduna State. The worms were collected in specimen bottles containing 0.9% physiological saline and taken to Helminthology Laboratory of the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The worms were crushed in distilled water using a mortar and pestle to release the eggs from the uteri of adult female. The crushed worms were then filtered out using a mesh of 0.01 mesh size into a beaker. The filtrate was then allowed to stand for about an hour after which the supernatant was decanted. The sediment was then washed with 0.5 M sodium hydroxide solution into a beaker and agitated gently for 30 minutes in order to dissolve the sticky albuminous layer of eggs and allowed for uniform sampling. This was then placed in centrifuge tubes and centrifuged at 23.25 xg per minute for 3 minutes to recover the eggs. The recovered eggs were then washed three times in distilled water and also three times in embryonating fluid which is a solution of 0.05 M sulfuric acid. The eggs collected were suspended in embryonating fluid and placed in plastic troughs. This was then left to stand for 21 days in the laboratory at 30°C. Embryonating fluid was periodically added to

the egg cultures to avoid drying. Embryonated eggs were then stored at room temperature for two weeks before infection of the birds (Hansen *et al.*, 1954; Fairbairn, 1970).

### **3.6 Experimental infection of Guinea Fowl Keets**

The method of Velkers *et al.* (2011) was used for the inoculation of the keets with embryonated eggs of *A. galli* at six weeks of age. The larvae viability was microscopically (light microscope) assessed by observing the larvae spontaneous movement inside the egg. Each bird in groups I-IV were orally infected with 700 viable *A. galli* eggs contained in 0.4 ml of normal saline in an insulin syringe (Adang *et al.*, 2011).

### **3.7 Experimental Design and Treatments**

At six weeks of age, the guinea fowl keets were randomly divided into 5 groups (I, II, III, IV and V) of twenty birds per group (Table 3.1) and inoculated with *A. galli* eggs at nine weeks of age (3 weeks post-infection and 3 days after the first detection of *A. galli* eggs), treatments begun based on a positive faecal eggcount of *A. galli* in keets in groups I – IV. Keets in group I were infected with *A. galli* and treated with 2,000mg/litre of concentrated Xafruit extract orally for 3 days. Group II were infected with *A. galli* and treated with 4,000 mg/litre of concentrated Xafruit extract for 3 days. Group III were infected with *A. galli* and given 1,000 mg/litre of piperazine orally for one day (positive control). Group IV were infected with *A. galli* and given plain water (negative control). Birds in Group V were uninfected and untreated (positive control group for baseline values).

**Table 3.1: Experimental design of guinea fowl keets experimentally infected with *Ascaridia galli* and treated with aqueous extract of *Xylopia aethiopica* whole fruits (groups I and II) and piperazine (group III) for three days.**

Group	No. of Keets	Infectionwith	Dose	Route	Duration of
		<i>A. galli</i> / Treatment			treatment (days)
I	20	Infected/ 2000 mg/litre <i>Xa</i>	8 g	Oral	3
II	20	Infected/ 4000 mg/litre <i>Xa</i>	16 g	Oral	3
III	20	Infected/ Piperazine (1000 mg/litre)	4 g	Oral	3
IV	20	Infected/Untreated	-	-	-
V	20	Uninfected/Untreated	-	-	-

Key: - = Untreated

### **3.8 Faecal Examination**

To test the development of adult worms and onset of egg shedding, pooled faecal samples were taken from the infected groups at 4 weeks post-infection and then every 2 days. Faecal samples were taken every day from the onset of the treatment up to seven days post treatment. Faecal egg count (FEC) was determined as the number of eggs per gram of faeces (EPG). Faecal Egg Count (FEC) was measured by a modified McMaster technique adopted from MAFF (1986).

### **3.9 Determination of Percentage Deparasitization**

The efficacy of the drug on the basis of postmortem worm count was evaluated according to the method described by Soulsby (1982):

$$AE = \frac{A - B}{A} \times 100 = (\%)$$

Where:

AE = Anthelmintic efficacy

A = Number of parasites in infected untreated birds.

B = Number of parasites in treated birds.

### **3.10 Evaluation of Haematological Parameters**

#### **3.10.1 Collection of blood**

Two millilitres of blood were collected using 5 ml syringe and 23 gauge needle from three birds chosen randomly in each group by wing venipuncture in the morning between 7am and 9am. Collection of blood was done at 6 weeks of age (before infection), 9 weeks of age (3 weeks post infection) and 10 weeks of age (3days post treatment). One millilitre out of the two millilitres of blood collected from each of the birds was allowed into sterile vials with 20 µl of 10%

ethylenediaminetetraacetic acid (EDTA), for haematology, while the remaining 1ml was transferred into another bottle without EDTA for serology. The blood without EDTA was allowed to clot, then serum was separated immediately by centrifugation at 16.24gpm for three minutes. The sera samples obtained were stored at -20°C until used.

Blood samples were quickly refrigerated at 4°C and analysed within 24 h. Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by the microhematocrit and cyanmethaemoglobin methods, respectively, as described by Jain (1986). Erythrocyte count was determined by the haematocytometry method as described by Jain (1986). Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from Giemsa stained slides viewed (Coles, 1989). Absolute differential leucocyte counts (DLC) namely; the neutrophil, eosinophil, lymphocyte, monocyte and basophil values were determined using standard methods (Coles, 1980; Jain, 1986).

### 3.10.2 Packed cell volume

Packed cell volume, which is a measure of the proportion of the volume of the whole blood that is occupied by red blood cells, was determined by the microhaematocrit centrifugation technique (Jain 1986). Blood in a sample vacutainer tube was mixed by gently inverting the tube about 20 times. The blood was drawn three quarters of the way up a 75 mm x 1.0 mm microhaematocrit capillary tube. Blood was wiped off the tip of the capillary tube, and the end of the capillary tube was carefully plugged with plasticine. The capillary tubes were placed, with the closed end outwards, in a microhaematocrit centrifuge (Hawksley & Sons Limited, England) and spun at



194.81 gpm for 5 min. The capillary tube was removed from the centrifuge, placed on a haematocrit reader and the PCV was recorded.

### 3.10.3 Haemoglobin concentration

Haemoglobin concentration was measured spectrophotometrically by the cyanmethaemoglobin method (Jain, 1986) in the Clinical Pathology Laboratory of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. Blood in a sample vacutainer tube was mixed gently by inverting about 20 times. Twenty microlitres of blood was added to 5 ml of Drabkin's solution (containing potassium ferricyanide and potassium cyanide) in a test tube. In the Drabkin's solution, the red blood cells would be haemolysed and the haemoglobin was oxidised by the ferricyanide to methaemoglobin. The cyanide would then convert the methaemoglobin to stable cyanmethaemoglobin. The mixture was allowed to stand for 15 min. After that, 1 ml of the mixture was pipetted into a cuvette. The cuvette was placed in a spectrophotometer (Jenway, England, Model: Genova MK3) set at 540 nm, and the absorbance of the cyanmethaemoglobin solution was read after zeroing the spectrophotometer using neutral Drabkin's solution. The haemoglobin concentration of the blood sample was calculated by dividing the absorbance value by the slope obtained from a calibration graph. To obtain the calibration graph, a standard blood sample (of known haemoglobin concentration) was diluted with Drabkin's solution: 5 in 0; 4 in 1; 3 in 2; 2 in 3 and 1 in 4. The absorbance of each of the five solutions was read in the spectrophotometer after the spectrophotometer was zeroed using neutral Drabkin's solution. A graph of absorbance for each of the five solutions was plotted against the corresponding haemoglobin concentration, and the slope of the graph was determined. The haemoglobin concentration of each of the five solutions was obtained by multiplying the proportion of standard haemoglobin in that solution with the haemoglobin concentration value of the standard.

### **3.11 Evaluation of Serum Biochemical Parameters**

Serum biochemistry was performed on samples obtained from the five groups at the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital, Nigeria. Serum levels of Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase and serum Albumin (ALB) were estimated using laboratory kits obtained from Randox Laboratory Ltd., United Kingdom and absorbance were read using a UV-VIS Spectrophotometer (DREL 300 HACH).

#### **3.11.1 Aspartate aminotransferase**

Aspartate aminotransferase (AST) catalyzes the transamination of aspartate to alpha-keto glutarate to form glutamate and oxaloacetate, which then reacts with 2,4-dinitro-phenylhydrazine to form the hydrazone derivative of oxaloacetate, a coloured complex was measurable at 546 nm. The activity (U/L) was determined from the standard calibration graph.

#### **3.11.2 Alanine aminotransferase**

Alanine transaminase (ALT) catalyzes the transamination of alanine to alpha-keto glutarate to form glutamate and pyruvic acid, which then reacts with 2,4-dinitro-phenylhydrazine to form the hydrazone derivative of pyruvate, a coloured complex which can be measured at 546 nm. The activity (U/L) was determined from the standard calibration graph.

#### **3.11.3 Alkaline phosphatase**

Alkaline phosphatase (ALP) acts on esters of phosphoric acid and catalyse hydrolysis and phosphate group release. P-nitrophenol phosphate was used as the substrate, a colorless solution

turned yellow upon hydrolysis. The yellow coloured complex was measured spectrophotometrically at 405 nm. Activity (U/L) =  $3300 \times \text{change in absorbance at } 405\text{nm} / \text{change in time (minutes)}$ .

#### 3.11.4 Serum albumin

Bromocresol green formed a colored complex specifically with albumin. The intensity of the color, measured at 620nm, is directly proportional to the albumin concentration in the sample. The concentration of total serum protein (g/dl) was determined from the calibration graph.

### 3.12 Data Analyses

Results of faecal egg count, worm count, haematological and biochemical parameters were expressed as mean  $\pm$  SEM. Significance difference between means of groups was determined by one-way analysis of variance (ANOVA). Student's t-test was also used to compare variables pre and post-treatment. Graphpad prism for windows version 1.5 (Graphpad software, San Diego, C.A, USA, 2007) was used for all statistical analysis. P value <0.05 was considered as significant.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Phytochemical constituents of aqueous extract *Xylopi aethiopica* whole fruits

The phytochemical analysis conducted on the aqueous extract of the *Xylopi aethiopica* whole fruits used in this research revealed the presence of saponins, tannins, alkaloids, triterpene, flavonoids, carbohydrates, and cardiac glycosides (Table 4.1).

#### 4.2 Acute Toxicity of *Xylopi aethiopica*

The acute toxicity test showed that concentrated *Xylopi aethiopica* aqueous whole fruits extract had LD<sub>50</sub> above 5,000mg/kg body weight.

#### 4.3 Changes in Faecal Egg Per Gram Count

*A. galli* eggs were detected in the keets 4 weeks post-infection (Plate III). There were significant reduction ( $p < 0.05$ ) in the mean egg per gram (EPG) count in all infected/treated groups I ( $3,870 \pm 35$  to  $2,140 \pm 68$ ), II ( $2,876 \pm 23$  to  $1,513 \pm 59$ ), III ( $3,435 \pm 30$  to  $306.7 \pm 13$ ) when compared to infected/untreated group IV where there was a significant increase ( $p < 0.05$ ) from  $4,251 \pm 39$  to  $10,233 \pm 14$ . There were also significant difference ( $p < 0.05$ ) in the EPG count between the infected/treated groups (Table 4.2).

**Table 4.1: Phytochemical components of aqueous extract of *Xylopi aethiopica* whole fruits obtained from Samaru market, Zaria, Nigeria.**

Constituents	Tests	Inferences
Carbohydrates	Molisch	+
Anthraquinone	Bontrongers	-
Cardiac glycosides	Keke- Killani	+
Saponins	Frothing test	+
Steroids	Liberman Buchard	-
Triterpene	Liberman Buchard	+
Tannins	Ferric Chloride	+
Flavonoids	Sodium hydroxide	+
Alkaloids	Dragendorffs	+

Keys: + = Present, - = Absent.



**Plate XIII: Embryonated eggs of *Ascaridia galli* (arrow) (x40 magnification) isolated from the uteri of adult female *Ascaridia galli* 21 days post incubation.**

**Table 4.2: Mean ( $\pm$  SE) egg per gram count of *Ascaridia galli* eggs in guinea fowl keets pre and post treatment with aqueous extract of *Xylopia aethiopic* whole fruits obtained from Samaru market, Zaria, Nigeria.**

Groups	Treatment	Dose	Mean egg per gram count 4 weeks post-infection (pre-treatment)	Mean egg per gram count 7 days post-treatment
I	Infected/ <i>Xa</i>	2,000 mg/l	$3,870 \pm 34^a$	$2,140 \pm 68^b$
II	Infected/ <i>Xa</i>	4,000 mg/l	$2,876 \pm 23^a$	$1,513 \pm 59^c$
III	Infected/Piperazine	1,000 mg/litre	$2,876 \pm 23^a$	$306.7 \pm 12^e$
IV	Infected/Untreated	-	$4,251 \pm 37^a$	$10,233 \pm 13^d$
V	Uninfected/Untreated	-	0	0

*Xa*: *Xylopia aethiopic*. Values in columns with different superscript are significantly different at  $p < 0.05$  (One-way ANOVA followed by Student t-test).

#### **4.4 Worm Count**

*A. galli* were seen in the intestines of the keets 4 weeks post-infection when postmortem worm count was carried out on three keets per group (Plates IV and V). The mean worm count showed significant reduction ( $p < 0.05$ ) 7 days post-treatment in the treated groups I ( $221 \pm 32$  to  $172 \pm 18$ ), II ( $205 \pm 18$  to  $129.3 \pm 32$ ), III ( $195 \pm 12$  to 0) when compared to the infected/untreated group IV where there was an increase from  $214 \pm 19$  to  $234 \pm 10$ ). There were also significant difference ( $p < 0.05$ ) in the mean postmortem worm count between the infected/treated groups 7 days post-treatment (Table 4.3).

#### **4.5 Percentage Deparasitization**

After 7 days post-treatment, the percentage deparasitization observed in groups I (Infected/*Xa* 200 mg/l), II (Infected/*Xa* 400 mg/l) and III (Infected/piperazine 15 mg/l) were 26.5%, 44.4% and 100%, respectively (Figure 4.1).

#### **4.6 Haematological Parameters**

From the results shown in table 4.4, there were increases in the mean PCV of infected/treated groups I ( $35.0 \pm 2.68$  to  $38.7 \pm 1.25$  %), II ( $34.7 \pm 1.03$  to  $38.2 \pm 1.03$ %) and III ( $37.0 \pm 0.91$  to  $40.0 \pm 2.27$ %) when compared to infected/untreated group IV ( $34.3 \pm 3.42$ %) 7 days post-treatment (Appendix I). Similarly, there were also increases in the mean Hb concentrations of infected/treated groups I ( $11.6 \pm 0.88$  to  $12.9 \pm 0.42$  g/dl), II ( $11.5 \pm 0.35$  to  $12.6 \pm 0.27$  g/dl) and



III ( $12.3 \pm 0.31$  to  $13.3 \pm 0.76$  g/dl) when compared to the value in infected/untreated group IV ( $11.4 \pm 1.23$  g/dl) 7 days post-treatment (Appendix II).

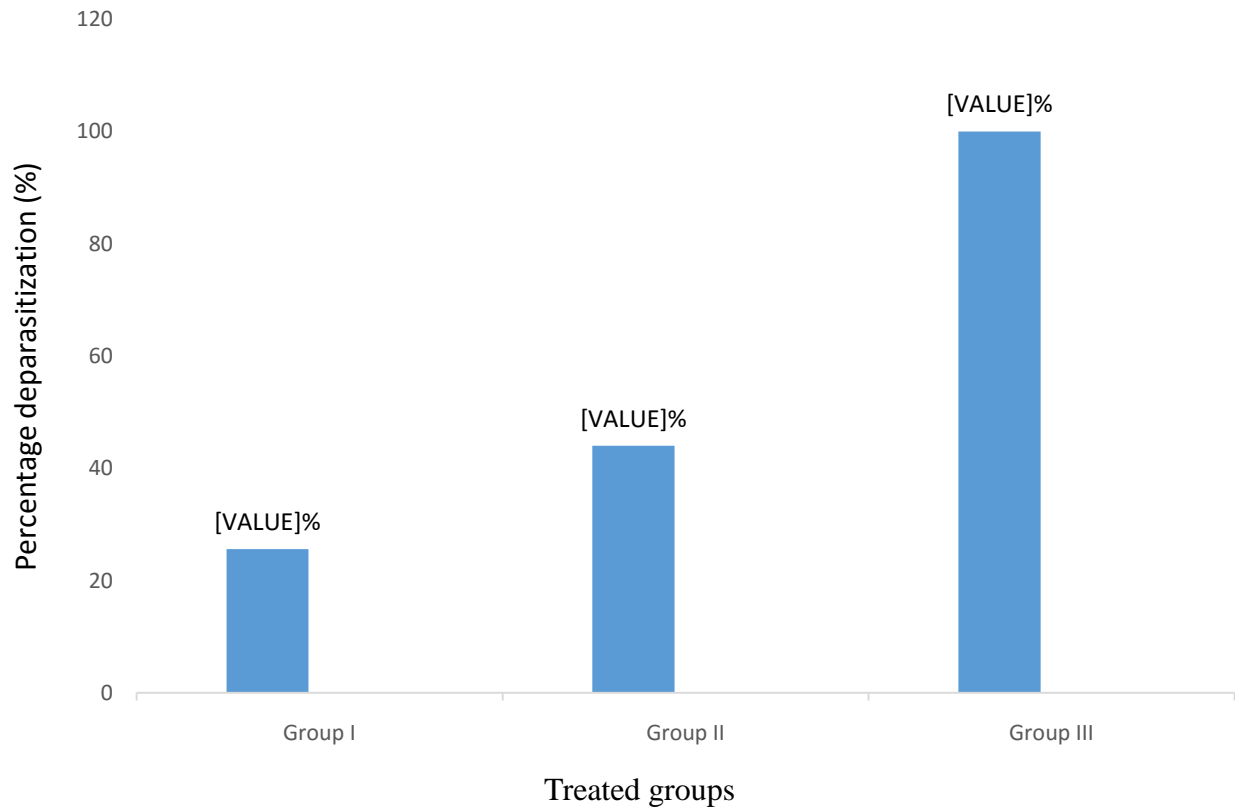


**Plate IX: *Ascaridia galli*(arrows) in the small intestines of a guinea fowl keet 4 weeks post-infection in group IV (infected and untreated).**

**Table 4.3: Mean ( $\pm$  SE) worm count of guinea fowl keets infected with *Ascaridia galli* one week post treatment with aqueous extract of *Xylopi aethiopic* whole fruits obtained from Samaru market, Zaria, Nigeria.**

Group	Treatment	Dose	Mean worm count 4 weeks post- infection (pre-treatment)	Mean worm count 7 days post-treatment
I	Infected/ <i>Xa</i>	2,000 mg/l	221 $\pm$ 32 <sup>a</sup>	172 $\pm$ 18.33 <sup>a</sup>
II	Infected/ <i>Xa</i>	4,000 mg/l	205 $\pm$ 18 <sup>a</sup>	129.3 $\pm$ 32.73 <sup>b</sup>
III	Infected/Piperazine	1,000mg/l	195 $\pm$ 12 <sup>a</sup>	0
IV	Infected/Untreated	-	214 $\pm$ 19 <sup>a</sup>	234 $\pm$ 10.79 <sup>c</sup>
V	Uninfected/Untreated	-	0	0

*Xa*: *Xylopi aethiopic*. Values are mean  $\pm$  Standard error of mean; Values in a column with a different superscript are significantly different ( $p < 0.05$ ) (One-way ANOVA followed by Student t-test).



**Figure 4.1:Percentage deparasitization in guinea fowl keets experimentally infected with *Ascaridia galli* and treated with aqueous extract of *Xylopia aethiopica* (groups I and II) and piperazine (group III) for three days.**

However, the RBC count were higher in groups I ( $5.8 \pm 0.45$  to  $6.8 \pm 0.25 \times 10^{12}/L$ ) and II ( $5.8 \pm 0.21$  to  $6.4 \pm 0.17 \times 10^{12}/L$ ). However, there were no significant increases in RBC count in groups III, IV and V. The WBC count were higher only in treated groups I ( $6.7 \pm 1.79$  to  $8.7 \pm 3.87 \times 10^9/L$ ) and II ( $7.4 \pm 1.70$  to  $9.6 \pm 2.05 \times 10^9/L$ ) (Table 4.4).

#### 4.7 Serum Biochemical Parameters

The results of some serum biochemical parameters of the keets infected with *Ascaridia galli* pre and post-treatment with *Xylopiia aethiopica* are presented in table 4.5. The mean (AST) level was higher in group IV ( $81.6 \pm 4.76$  U/l) when compared to the values in groups I ( $67.2 \pm 7.12$  U/L), II ( $74 \pm 9.13$  U/L), III ( $71.4 \pm 6.21$  U/L) and V ( $69 \pm 4.16$  U/L) (Appendix III).

The mean ALT levels were higher in groups III ( $5.2 \pm 0.20$  U/L) and IV ( $4.4 \pm 0.87$  U/L) when compared to the values in groups I ( $3.8 \pm 0.37$  U/L), II ( $3.6 \pm 0.24$  U/L) and V ( $3.4 \pm 0.24$  U/L) post-treatment (Appendix IV).

Higher ALP level were observed in groups I ( $540 \pm 94.79$  U/L) and IV ( $360.4 \pm 49.98$  U/L) when compared to the values in groups II ( $233.4 \pm 3.97$  U/L), III ( $331 \pm 38.46$  U/L) and V ( $187.8 \pm 25.80$  U/L) post-treatment (Appendix V).

The serum ALB level was lower in group IV ( $3.2 \pm 3.20$  U/L) when compared to the values in groups I ( $37.6 \pm 24.50$  U/L), II ( $06.4 \pm 3.92$  U/L), III ( $27.2 \pm 8.62$  U/L) and baseline values in V ( $12.8 \pm 3.20$  U/L) post-treatment (Appendix VI).

However, there was higher plasma proteins in groups I ( $3.6 \pm 0.12$  to  $4.6 \pm 0.46$  g/dl) and III ( $3.6 \pm 0.31$  to  $5.1 \pm 0.58$  g/dl), but decreased in group II ( $6.0 \pm 2.45$  to  $4.6 \pm 0.47$  g/dl) (Table 4.5, Appendix VII).

**Table 4.4: Mean ( $\pm$  SE) haematological parameters of guinea fowl keets experimentally infected with *Ascaridia galli* pre and post-treatment with aqueous extract of *Xylopia aethiopica*(Xa)whole fruits obtained from Samaru market, Zaria, Nigeria.**

Parameters		Group I	Group II	Group III	Group IV	Group V
PCV (%)	Pre-Treatment	35.0 $\pm$ 2.68 <sup>a</sup>	34.7 $\pm$ 1.03 <sup>a</sup>	37.0 $\pm$ 0.91 <sup>a</sup>	34.0 $\pm$ 1.32 <sup>a</sup>	37.3 $\pm$ 2.15 <sup>a</sup>
	Post-Treatment	38.7 $\pm$ 1.25 <sup>a</sup>	38.2 $\pm$ 1.03 <sup>a</sup>	40.0 $\pm$ 2.27 <sup>a</sup>	34.3 $\pm$ 3.42 <sup>a</sup>	37.4 $\pm$ 2.68 <sup>a</sup>
Hb (g/dL)	Pre-Treatment	11.6 $\pm$ 0.88 <sup>a</sup>	11.5 $\pm$ 0.35 <sup>a</sup>	12.3 $\pm$ 0.31 <sup>a</sup>	11.2 $\pm$ 1.87 <sup>a</sup>	12.3 $\pm$ 0.88 <sup>a</sup>
	Post-Treatment	12.9 $\pm$ 0.42 <sup>a</sup>	12.6 $\pm$ 0.27 <sup>a</sup>	13.3 $\pm$ 0.76 <sup>a</sup>	11.4 $\pm$ 1.23 <sup>a</sup>	12.5 $\pm$ 0.97 <sup>a</sup>
RBC (x 10 <sup>12</sup> /L)	Pre-Treatment	5.8 $\pm$ 0.45 <sup>a</sup>	5.8 $\pm$ 0.21 <sup>a</sup>	6.3 $\pm$ 0.25 <sup>a</sup>	5.6 $\pm$ 0.57 <sup>a</sup>	6.3 $\pm$ 0.72 <sup>a</sup>
	Post-Treatment	6.8 $\pm$ 0.25 <sup>a</sup>	6.4 $\pm$ 0.17 <sup>a</sup>	6.9 $\pm$ 0.11 <sup>a</sup>	5.6 $\pm$ 0.89 <sup>a</sup>	6.6 $\pm$ 0.32 <sup>a</sup>
WBC (x 10 <sup>9</sup> /L)	Pre-Treatment	6.7 $\pm$ 1.79 <sup>a</sup>	7.4 $\pm$ 1.70 <sup>a</sup>	9.2 $\pm$ 3.60 <sup>a</sup>	5.2 $\pm$ 1.43 <sup>a</sup>	4.7 $\pm$ 0.70 <sup>a</sup>
	Post-Treatment	8.7 $\pm$ 3.87 <sup>a</sup>	9.6 $\pm$ 2.05 <sup>a</sup>	9.5 $\pm$ 3.37 <sup>a</sup>	5.8 $\pm$ 1.46 <sup>a</sup>	4.9 $\pm$ 1.90 <sup>a</sup>

PCV (Packed cell volume), Hb (Haemoglobin), RBC (Red blood Cell), WBC (White Blood Cells).p <0.05 significant.Values are mean  $\pm$  Standard error of mean; Values in a row with a different superscript are significantly different (p<0.05).

Group I: 2,000mg/l (Xa), Group II: 4,000mg/l (Xa), Group III: 1,000 mg/l Piperazine, Group IV: Infected/Untreated, Group V: Uninfected/Untreated.(One-way ANOVA followed by Student t-test).

**Table 4.5: Mean ( $\pm$  SE) serum biochemical parameters of guinea fowl keets experimentally infected with *Ascaridia galli* pre and post-treatment with aqueous extract of *Xylopia aethiopica*(Xa) whole fruits obtained from Samaru market, Zaria, Nigeria.**

Parameters		Group I	Group II	Group III	Group IV	Group V
AST (U/l)	Pre-Treatment	96.6 $\pm$ 23.95 <sup>a</sup>	84 $\pm$ 13.23 <sup>a</sup>	66.6 $\pm$ 14.1 <sup>a</sup>	80.6 $\pm$ 14.34 <sup>a</sup>	67.3 $\pm$ 4.91 <sup>a</sup>
	Post-Treatment	67.2 $\pm$ 7.12 <sup>a</sup>	74 $\pm$ 09.13 <sup>a</sup>	71.4 $\pm$ 6.21 <sup>a</sup>	81.6 $\pm$ 04.76 <sup>a</sup>	69.0 $\pm$ 4.16 <sup>a</sup>
ALT (U/l)	Pre-Treatment	3.3 $\pm$ 0.33 <sup>a</sup>	4.3 $\pm$ 0.88 <sup>a</sup>	3.6 $\pm$ 0.67 <sup>a</sup>	4 $\pm$ 0.58 <sup>a</sup>	3 $\pm$ 0 <sup>a</sup>
	Post-Treatment	3.8 $\pm$ 0.37 <sup>a</sup>	3.6 $\pm$ 0.24 <sup>a</sup>	5.2 $\pm$ 0.20 <sup>a</sup>	4.4 $\pm$ 0.87 <sup>a</sup>	3.4 $\pm$ 0.24 <sup>a</sup>
ALP (U/l)	Pre-Treatment	226.7 $\pm$ 32.84 <sup>a</sup>	391.3 $\pm$ 42.1 <sup>a</sup>	291.7 $\pm$ 37.86 <sup>a</sup>	286.3 $\pm$ 92.07 <sup>a</sup>	191.3 $\pm$ 14.67 <sup>a</sup>
	Post-Treatment	540 $\pm$ 94.79 <sup>a</sup>	233.4 $\pm$ 3.97 <sup>b</sup>	331 $\pm$ 38.46 <sup>a</sup>	360.4 $\pm$ 49.98 <sup>a</sup>	187.8 $\pm$ 25.80 <sup>b</sup>
PP (g/dL)	Pre-Treatment	3.6 $\pm$ 0.12 <sup>a</sup>	6.0 $\pm$ 2.45 <sup>a</sup>	3.6 $\pm$ 0.31 <sup>a</sup>	3.1 $\pm$ 0.41 <sup>a</sup>	3.5 $\pm$ 1.21 <sup>a</sup>
	Post-Treatment	4.6 $\pm$ 0.46 <sup>a</sup>	4.6 $\pm$ 0.47 <sup>a</sup>	5.1 $\pm$ 0.58 <sup>a</sup>	3.1 $\pm$ 0.49 <sup>a</sup>	3.6 $\pm$ 0.29 <sup>a</sup>

AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alanine phosphatase), TP (Total protein), ALB (Albumin) PP (Plasma Protein). Values are mean  $\pm$  Standard error of mean; Values in a row with a different superscript are significantly different (p<0.05).

Group I: 2,000 mg/l (Xa), Group II: 4,000 mg/l (Xa), Group III: 1,000 mg/l Piperazine, Group IV: Infected/Untreated, Group V: Uninfected/Untreated. (One-way ANOVA followed by Student t-test).

## CHAPTER FIVE

### 5.0 DISCUSSION

The phytochemical analyses conducted on the crude aqueous extract of the *Xylopia aethiopica* used in this research revealed the presence of saponins, tannins, alkaloids, triterpene, flavonoids, carbohydrates, and cardiac glycosides. Phytochemicals, such as tannins, alkaloids, flavonoids and saponins have been shown to possess anthelmintic activities (Oladele *et al.*, 1995). It could be possible that the tannins present in *Xylopia aethiopica* in this study contain extracts which produced similar effects as phenolic anthelmintics. It has been reported that tannins can bind to free proteins in the gastrointestinal tract of the host animal as glycoprotein on the cuticle of the parasite and cause its death as reported by Thompson and Geary (1995). Also alkaloids can act on the central nervous system of the earthworms causing paralysis as reported by Roy (2010).

The anthelmintic effect of *Xylopia aethiopica* observed in this study could also be due to presence of the steroidal alkaloids oligosaccharides which have been reported to suppress the transfer of sucrose from the stomach to the small intestine which could diminish the availability of glucose to helminthes (Roy, 2010). The extracts might have also induced possible inflammatory effect in the gastric and intestinal mucosa which could have caused increased peristalsis leading to expunging of the parasites (Ekeanyanwu and Etienajirhevwe, 2012).

The main biologic activity ascribed to saponins based on recent research is their membrane permeability property (Wang, 2010). The main possible actions of saponins are changes in membrane permeability and pore formation, similar with two conventional anthelmintic drugs



such as praziquantel. It could be that the *Xylopiya aethiopica* whole fruits aqueous extract used in this research was able to produce similar effects.

Piperazine when used at 1,000 mg/l was found to have 100% deparasitization compared to *Xylopiya aethiopica* which had 26.5% and 44.4% deparasitization at doses of 2g/l and 4g/l, respectively. The finding complements a previous report that piperazine is one of the most potent anthelmintic drugs (Rehman *et al.*, 2014). The piperazine salts are potent anthelmintics which act as pharmacological analogue of a natural inhibitory neurohormone (Rehman *et al.*, 2014). Different scientists have reported different efficacies of piperazine against *Ascaridia galli* in birds. Pavlíček and Dyková (1976) reported that efficacy of piperazine ranged from 17-67%, while Ashraf (1980) stated that Piperazine was 100% effective against mature and 72% for immature *Ascaridia galli* worms. Similarly, Sharif (1980) reported that piperazine was 100% efficacious against adult *Ascaridia galli*. Sharma (1990) administered piperazine dihydrochloride, at dosage of 64, 80 and 100 mg/kg body weight and reported its efficacy against adult *Ascaridia galli* as 83%, 94% and 100%, respectively. Ihsanullah (1999) reported that piperazine was 88.66% efficient at day 7 and 99.31% at day 14 post treatment.

The percentage deparasitisation observed in group I was found to be 26.5% compared to group II and III which had 44.4% and 100% respectively. Suleiman *et al.* (2005) reported that for an anthelmintic to be considered effective, it has to reduce 50% or more of worm burden in an animal. Even though, the *Xylopiya aethiopica* concentrated aqueous fruit extract used in this study could not produce 50% percentage deparasitization, it was found to produce some degree of anthelmintic activity which was found to be concentration dependent. This findings agrees with

the findings of Suleiman *et al.* (2005) who found out that *Xylopi aethiopica* fruits extract was able to produce a concentration dependent anthelmintic effect on *Nippostrongylus brasiliensis* in rats. However the percentage deparasitisation produced by the *Xylopi aethiopica* in this study (26.5% and 44.4% at doses of 2 gram/l and 4 gram/l respectively) was below the one observed by Suleiman *et al.* (2005) in rats (63% and 76% at a doses of 1.4 gram/kg and 1.7 gram/kg respectively). The low anthelmintic activity observed in this study might be due to the low concentration of the phytochemical compounds obtained from the concentrated aqueous extract used. It could also be due to variation in susceptibility of the parasites (*Ascaridia galli* in this study and *Nippostrongylus brasiliensis* used by Suleiman *et al.* (2005)) to *Xylopi aethiopica*.

The *Xylopi aethiopica* extract also caused increase in the levels of Hb, RBC and WBC count in the treated groups. This increase could be attributed to the anthelmintic effect of *Xylopi aethiopica* on the *Ascaridia galli* parasite as also observed in the group treated with piperazine. Increase in these parameters could also be due to a direct effect of the extract on haemopoietic activity in these keets as reported by Abaidoo *et al.* (2011). According to Eteng *et al.* (2012) alkaloid, which is one of the phytochemical components of *Xylopi aethiopica*, is known to cause similar effect by inhibiting phosphodiesterase leading to the accumulation of cAMP which in turn stimulates protein synthesis. Locally in Ghana, decoction of dry fruits of *Xylopi aethiopica* is taken as a postpartum tonic as well as an immune booster in individuals with low immunity (Burkill, 1985). Neutrophils are the main type of white blood cells that are responsible for fighting infectious agents by phagocytosis. Antimicrobial activity of *Xylopi aethiopica* has since been reported (Fleischer *et al.*, 2008). The findings of this study agrees with the work

of Idowu *et al.* (2009), who found that aqueous extract of *Xylopia aethiopica* was able to significantly increase the levels of Hb, PCV, WBC and Neutrophil in rats.

It has been reported that elevated levels of AST, ALT, ALP and GGT are indicative of cellular leakage and loss of functional integrity of hepatic cell membranes, implying hepatocellular damage (Chung *et al.*, 2001; Gupta *et al.*, 2007). The finding that *Xylopia aethiopica* extract was able to reduce the activity of AST, ALT and ALP when administered at a concentration of 4,000 mg/l (in comparison to the severity of the effect of the *Ascaridia galli* in the untreated keets) could be as a result of the repairing and protective ability of the phenolic compounds (Vijyan *et al.*, 2003) present in the *Xylopia aethiopica* fruit extract. The reparation of the damages in the liver by *Xylopia aethiopica* fruit extract has been attributed to its complementary effect in stimulating protein synthesis, biogenesis and as antioxidant (Lee, 2003). Further investigation into the physicochemical properties of this fruit extract, among other medicinal plants, revealed that it also has a high degree of unsaturated fatty acid components such as omega 3 and 6, which this may enable it to stabilize membrane as stated in the work of Ezekwesili *et al.* (2010). The presence of phenolic compounds and oxygenated volatile compounds such as cis-linalool oxide, Carveol, buten-1-ol, iso-borneol, Borneol, thymol, beta-citronellol, geraniol, dihydrocarveol, linalool in *Xylopia aethiopica* extract have made it to have strong antioxidant activity (Nadia *et al.*, 2013). These strong antioxidants might be responsible for the observed anti-hepatotoxic potential of *Xylopia aethiopica* extract (Nadia *et al.*, 2013). On the basis of the fact that the phenolic compounds and terpenes are known for their properties to trap the free radicals (Jukić and Milos, 2005), it is well documented that polyphenols are good hepatoprotective agents because they can effectively inhibit lipid peroxidation, scavenge free radicals and enhance

antioxidant enzyme activities (Weiss and Landauer, 2003), just as well as it is known that oxygenated volatile compounds exhibited a high antioxidant power (Abd El Mageed *et al.*, 2012).

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

From this work, it was concluded that:

- i. *Xylopi aethiopica* at concentrations of 2,000 mg/l and 4,000 mg/l after three days of treatment has antihelminthic effect of 26.5%, and 44.4% respectively.
- ii. *Xylopi aethiopica* when administered at 4,000 mg/l caused increase in packed cell volume (from  $34.7 \pm 1.03\%$  to  $38.2 \pm 1.03\%$ ), haemoglobin concentration (from  $11.5 \pm 0.35\text{g/dl}$  to  $12.6 \pm 0.27\text{g/dl}$ ) and red blood cell count (from  $5.8 \pm 0.21 \times 10^{12}/\text{L}$  to  $6.4 \pm 0.17 \times 10^{12}/\text{L}$ ) in keets experimentally infected with *A. galli*.
- iii. *Xylopi aethiopica* when administered at 4,000mg/l was able to cause decrease in serum aspartate aminotransferase (from  $84 \pm 13.23\text{u/l}$  to  $74 \pm 09.13\text{u/l}$ ), alanine aminotransferase (from  $4.3 \pm 0.88\text{u/l}$  to  $3.6 \pm 0.24\text{u/l}$ ) and alkaline phosphatase (from  $391.3 \pm 42.1\text{u/l}$  to  $233.4 \pm 3.97\text{u/l}$ ) in treated keets infected with *A. galli*.

### **6.3 Recommendations**

- i. From the results of these study, the use of aqueous extract of *Xylopi aethiopica* whole fruits as an anthelmintic remedy especially in rural poultry is recommended.
- ii. Further studies to identify the most effective non-toxic dose of *Xylopi aethiopica* in relation to its other parts (bark, stem, roots and leaves) for the treatment of helminthes need to be conducted.
- iii. Study of the extract using many more doses and changing the method of administration need to be conducted.

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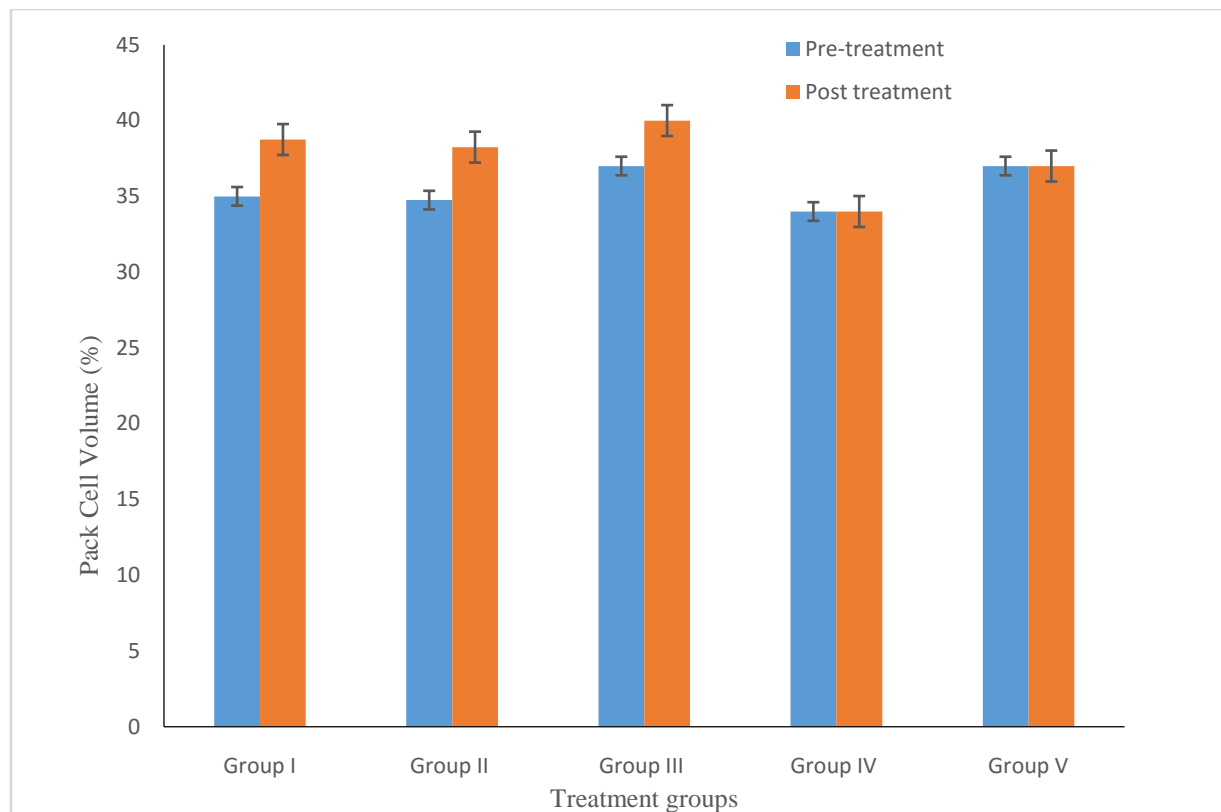
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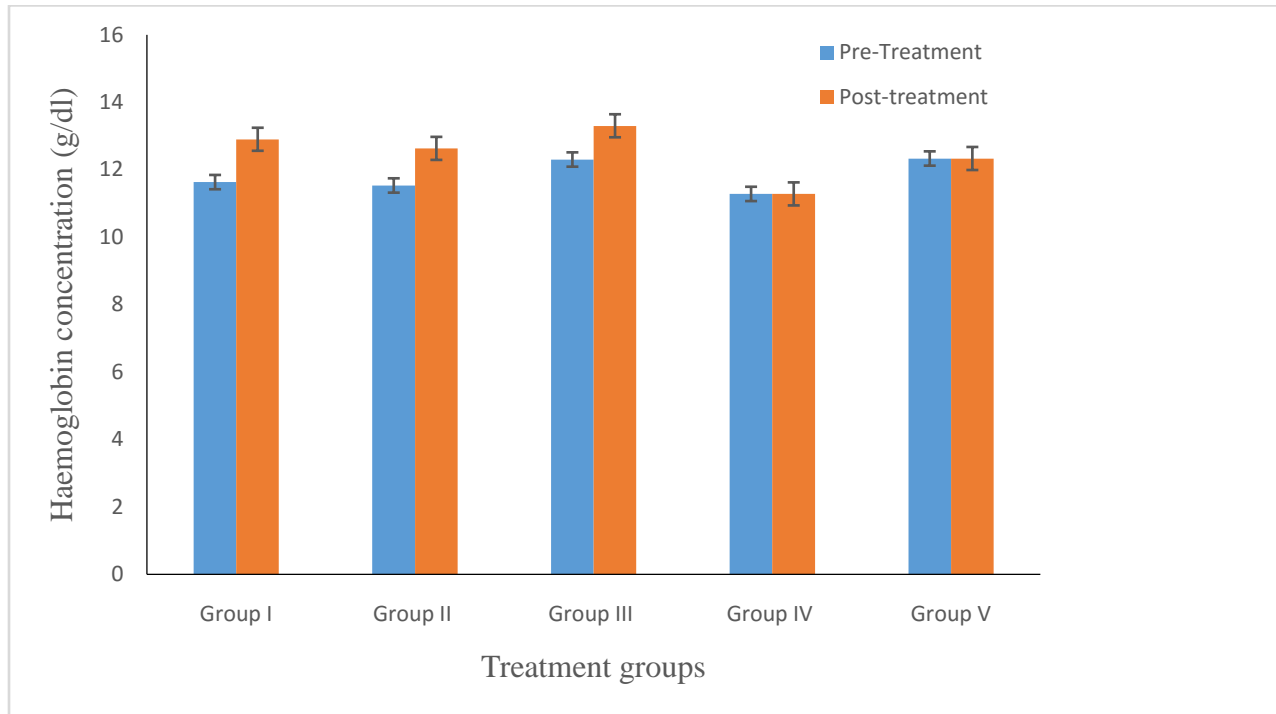
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## APPENDICES

**Appendix I: Mean ( $\pm$  SE) packed cell volume of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopia aethiopica*.**

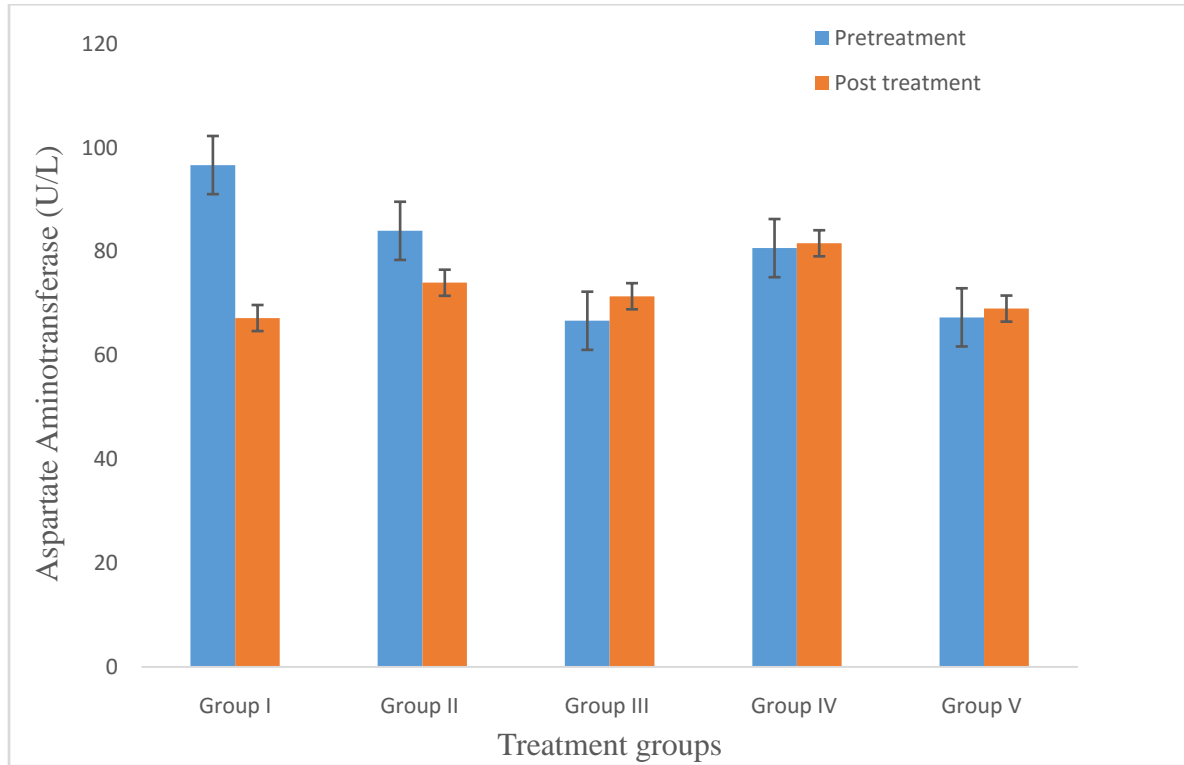


**Appendix II: Mean ( $\pm$  SE) haemoglobin concentration of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopi aethiopica*.**

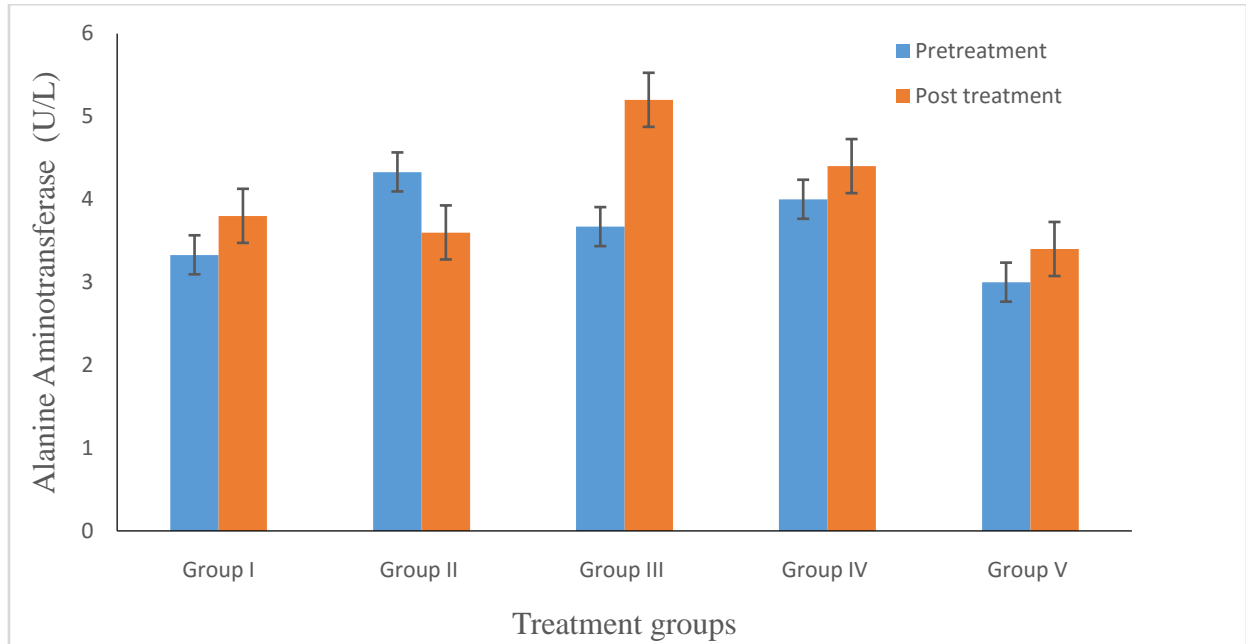




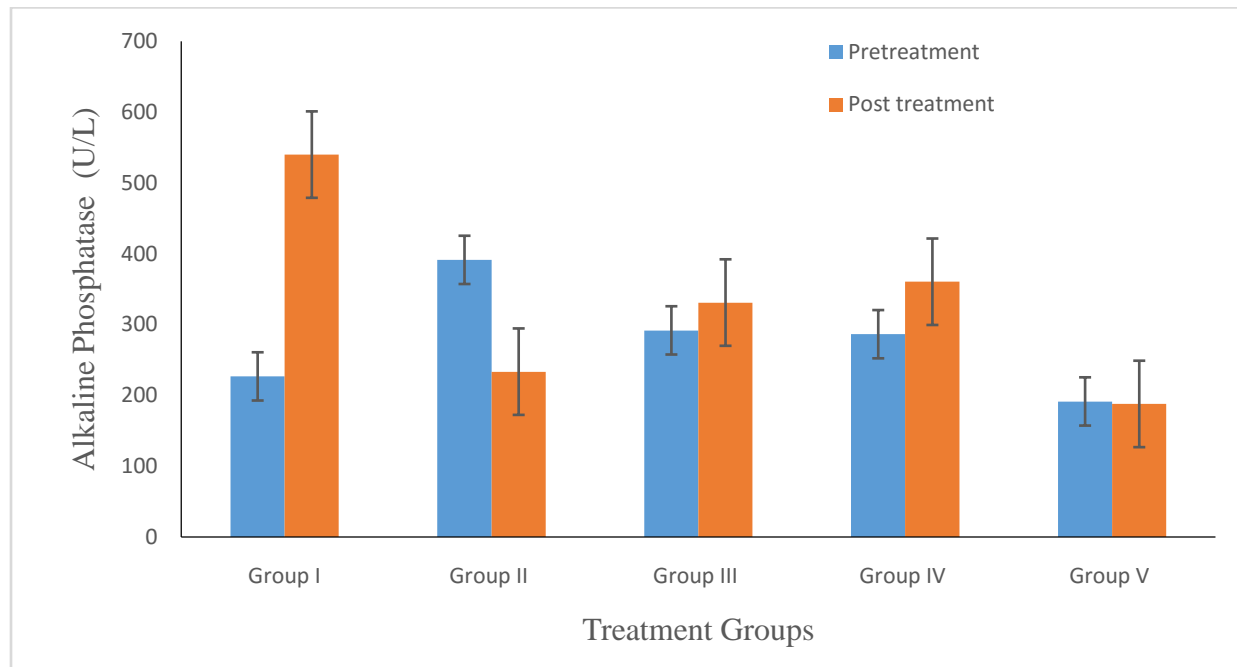
**Appendix III: Mean ( $\pm$  SE) serum aspartate aminotransferase (AST) level of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopia aethiopica*.**



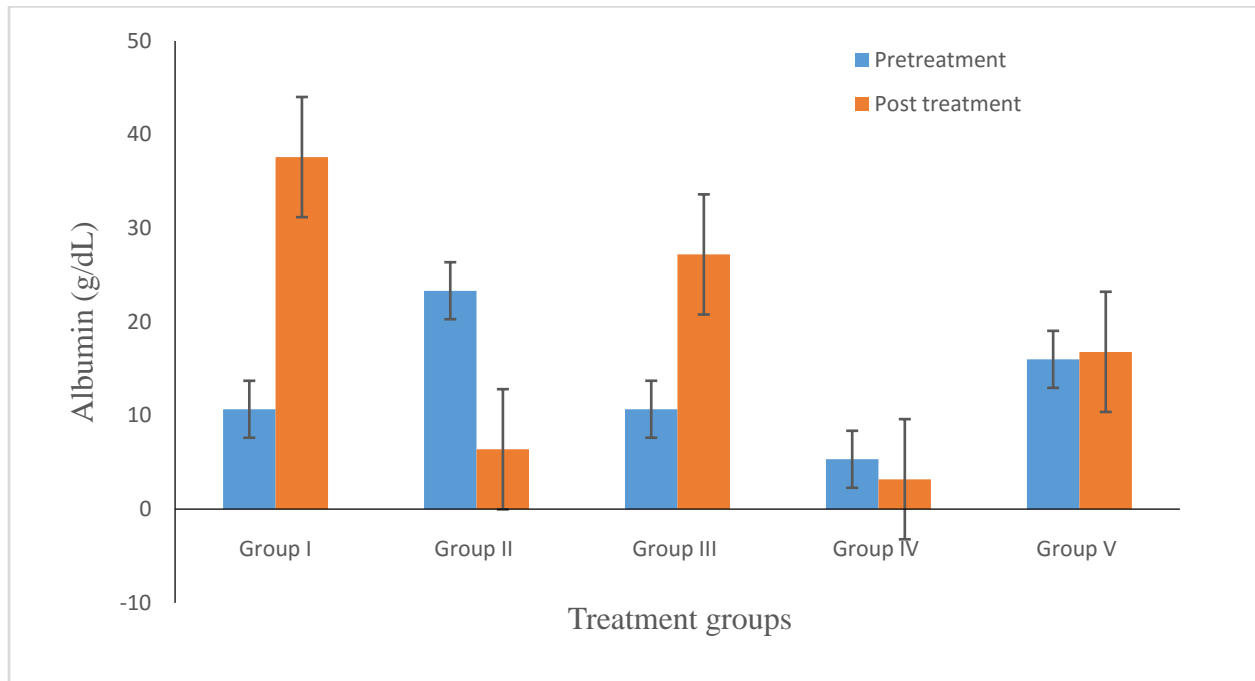
**Appendix IV: Mean ( $\pm$  SE) serum alanine aminotransferase (ALT) level of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopia aethiopica*.**



**Appendix V: Mean ( $\pm$  SE) serum alkaline phosphatase (ALP) level of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopi* *aethiopica*.**



**Appendix VI: Mean ( $\pm$  SE) serum albumin level of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopi aethiopica*.**



**Appendix VII: Mean ( $\pm$  SE) plasma proteins of guinea fowl keets infected with *Ascaridia galli* pre and post-treatment with *Xylopi aethiopica*.**

