COMPARISON OF THE EFFECTS OF BIOACTIVE COMPOUNDS OF PLANT PARTS OF *FICUS SYCOMORUS* AND *PILIOSTIGIMA THONNINGII* ON THE PERFORMANCE OF BROILERS

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THE UNIVERSITY OF ZAMBIA

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DECLARATION

I, Kabunda M. Grendah, declare that this Bachelor of Science in Agriculture Science (BSc. Agric) thesis represents my own work and that it has not previously been submitted for a degree at this or any other university. All sources of information have been acknowledged by citations and references.

Signature: .... Date: 12.09.2013

DEDICATION

To my father Rodrick Kabunda, mother Prisca Kabunda, Brother Isaac and sisters Bethar and Patience; in fond memory of my late grandmother Asa Chonde Chungu. To the Almighty God for His grace

ABSTRACT

The research aimed at comparing the effectiveness of bioactive compounds of edible plant parts of *P. thonningii* (leaves, fruit pod and bark) and *F. sycomorus (leaves and bark)* on growth, feed consumption and feed conversion ratio, of Cobb 500 broilers as potential replacements of synthetic growth promoting antibiotics. The experiment was conducted at the University of Zambia; School of Agricultural Sciences Field Station over a period of six (6) weeks in a Complete Randomized Block Design (CRBD), with 6 treatments and 3 replications. The 1% inclusion of *F.sycomorus* and *P. thonningii* plant parts in feed rations showed no significant differences ($P<0.05$) on the live weight, feed consumption, and feed conversion ratio in comparison to the birds on conventional management. The bioactive compounds in the edible plant parts of F. sycomorus and P. thonningii had a positive effect on the performance of broilers and hence may substitute for synthetic antibiotics.

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ABBREVIATIONS AND ACRONYMS

AOAC	American Oil Chemists' Society
Ash (%)	Percent Mineral Content
C.V	Coefficient of Variation
Ca (%)	Percent Calcium Content
Con.	Control treatment
CP (%)	Percent Crude Protein
DM (%)	Percent Dry Matter
EE (%)	Percent Ether Extract (Crude Oil)
FCR	Food Conversion Ratio
M (%)	Percent Moisture Content
Muk B.	*F. sycomorus* (Mukuyu) Bark treatment
Muk L.	*F. sycomorus* (Mukuyu) Leaf treatment
Mus B	*P. thonningii* (Musekese) Bark treatment
Mus F	*P. thonningii* (Musekese) Fruit treatment
Mus L	*P. thonningii* (Musekese) Leaf treatment
NARMS	National Antimicrobial Resistance Monitoring System
P (%)	Percent Phosphorus Content
R.E	Relative Efficiency of using RCBD over CRD
RCBD	Randomized Complete Block Design
RCD	Randomized Block Design

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CHAPTER 1

1.1 INTRODUCTION

Broiler production is the rearing of chickens for their meat; broiler birds have been selected and bred specifically for meat production. Broilers are important worldwide as sources of food protein and income. Under good management, broilers grow very fast and are ready for sale at about five or six weeks of age *(Farrell, 2000)*.

Benefits of the broiler industry include improving food self-sufficiency and alleviating malnutrition as it provides an excellent protein source (meat and eggs), minerals and vitamins which are essential for growth and maintenance of the body. It also creates employment generating activities (*Wethli, 1999; Farrell, 2000*) as a lot of labor is employed. Broilers can be sold or bartered to meet essential family needs such as medicine, clothes and school fees (*Alders, 2004; Alexander et al., 2004*). Approximately 20% of the protein consumed in developing countries comes from poultry meat and eggs. Rural household poultry production contributes 70% of total production in most low income, food-deficit countries (*Branckaert et al., 2000*).

1.1.1 BACKGROUND TO USE OF ANTIBIOTICS

For many years, the poultry industry has been looking for improvement of productivity and broiler growth through breeding changes in detriment of the final quality of products. Many factors may lead to alterations in meat quality, with those directly related being genetic (i.e. bird age, strain and sex) and environmental (i.e. nutrition, health and pre- and post-slaughter practices) (*Ferket, 2004*). Within the latter, the most directly related to alterations in meat quality is the use of antibiotics which have been particularly criticized by international health institutes, such as the Food and Drug Administration (FDA). However, their utility as a preventive or curative measure has been questioned, given extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria. Therefore, the possibility of antibiotics ceasing to be used as growth stimulants for poultry and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumer and manufacturer are looking for alternatives (Bedford, 2000).

1.1.2 THE BIOCHEMI CAL MECHANISMS OF ACTION OF ANTIBIOTICS AND THE R EFFECT ON GROWTH PROMOTION

One major aim of bioche mistry is to understand metabolism well enough to predict and control changes that occ r in cells. Biochemical studies have yielded such benefits as treatments for many metal olic diseases through the scientific breakthrough of antibiotics to deal with the negative effects of bacteria and hence boost industrial and agricultural productivity (*Bedford, 200)*).

Antibiotics (Greek anti, ' against"; bios, "life") are chemical compounds used to kill or inhibit the growth of infe tious organisms. Originally the term antibiotic referred only to organic compounds, produ ed by bacteria or molds, which are toxic to other microorganisms, (*Bedford, 2000*). The term s now used loosely to include synthetic and semisynthetic organic compounds. Antibiotic refe rs generally to antibacterials.

All antibiotics share the p operty of **selective toxicity**: They are more toxic to an invading organism than they are to a n animal or human host, (*Bedford, 2000*). Most antibiotics act by selectively interfering with the synthesis of one of the large-molecule constituents of the cell (the cell wall or proteins or nucleic acids). Some, however, act by disrupting the cell membrane (*Cell Death ana Growth Suppression*). Some important and clinically useful drugs interfere with the synthesis of peptidoglycan, the most important component of the cell wall. These drugs include the β-lactam antibiotics, which are classified according to chemical structure into *penicillins*, *cephalosporins*, and *carbapenems* and are commonly called *Bactericidal* antibiotics, inl ibit cell wall formation. All these antibiotics contain a β-lactam ring as a critical part of the r chemical structure. They inhibit synthesis of peptidoglycan, an essential part of the cell wa ll. They do not interfere with the synthesis of other intracellular components. The continuin g buildup of materials inside the cell exerts ever greater pressure on the membrane, which is no longer properly supported by peptidoglycan. The membrane gives way, the cell contents leak out, and the bacterium dies. These *antibiotics do not affect human cells because human cells do not have cell walls.*

The majority of antibiotics operate by inhibiting the synthesis of various intracellular bacterial molecules, includi g Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA), ribosomes, and proteins. Gro ups of antibiotics classified as *Bacteriostatic* bind with bacterial ribosomes to inhibit bacterial protein synthesis. Synthetic *sulfonamides* and *macrolides* are among the antibiotics that indirectly interfere with nucleic acid synthesis. Nucleic-acid synthesis can also be stopped by antibiotics that inhibit the enzymes that assemble these polymers (DNA polymerase or RNA polymerase). Examples of such antibiotics are *actinomycin*, *rifamicin*, and *rifampicin* (*Bedford, 2000*)

The antibiotics classified as *Quinolone* inhibit synthesis of an enzyme responsible for the coiling and uncoiling of the chromosome, a process necessary for DNA replication and for transcription to messenger RNA. These antibacterials (*tetracyclines*, *chloramphenicol*, *puromycins* and *aminoglycosides)* affect the assembly of messenger RNA, thus causing its genetic message to be garbled. When these faulty messages are translated, the protein products are nonfunctional. The *tetracyclines* mechanism of action is to compete with incoming transfer-RNA molecules; while the *aminoglycosides* cause the genetic message to be misread and a defective protein to be produced; *chloramphenicols* prevents the linking of amino acids to the growing protein; and *puromycin*s causes the protein chain to terminate prematurely, releasing an incomplete protein (*Bedford, 2000)*.

1.1.3 EFFECTS OF ANTIBIOTIC USE

Indiscriminate and inappropriate use of antibiotics for the treatment of the common cold and other common viral infections, against which they have no effect, removes antibiotic-sensitive bacteria and allows the development of antibiotic-resistant bacteria. Similarly, the use of antibiotics in poultry and livestock feed has promoted the spread of drug resistance and has led to the widespread contamination of meat and poultry by drug-resistant bacteria such as Salmonella *(Holmberg et al., 1984)*.

Furthermore, the use of antibiotics at sub-therapeutic doses which have been widely used in animal feed as growth promoters is amidst the concerns from consumers and other health practitioners. This is because in the presence of low levels of antibiotics, resistant cells of disease causing bacteria survive and grow producing an antibiotic resistant population in the final product. Therefore, the application of antibiotics as growth promoters in the animal feed has been limited in the European Union (EU) January 1996 (*Bedford, 2000*). As a result, new commercial feed additives of plant origin considered to be natural products that consumers would accept, have been proposed to livestock producers *(Bedford, 2000)*.

Broilers have routinely been fed antibiotics as "growth promoters" despite concerns that this could increase the risk of people developing antibiotic-resistant diseases *(Bedford, 2000)*. According to the Public Health Agency of Canada, *PHAC (2008)*, raw chicken remains the commodity most frequently contaminated with Salmonella and Campylobacter bacteria. Estimates vary for the prevalence of infected retail chicken meat, but surveillance by *PHAC (2008)* suggests it could be as high as 30 per cent for Salmonella and 43 per cent for Campylobacter. Salmonella can cause stomach cramps, diarrhea, vomiting and fever and can cause serious illness in infants, the elderly and people who are immune-compromised. Campylobacter can cause similar symptoms. One class of antibiotics, cephalosporins (e.g. Penicillin), are used to treat especially severe human infections. In the National Antimicrobial Resistance Monitoring System, NARMS, the use of cephalosporins was linked to human resistance to those antibiotics *(NARMS, 2005)* while the *PHAC (2008)* has stated that a growing body of evidence suggests that the clinical use of cephalosporins in human beings is under threat because of their widespread use in the poultry industry.

Breeding chickens solely for faster growth and more meat through the use of growth promoters, instead of for health and strong immune systems, has created an opportunity for diseases like avian flu to spread like wildfire through poultry populations (*Alexander et al., 2004*). There is thus a need to develop more natural remedies for disease prevention and control.

1.1.4 MECHANISMS OF BACTERIAL RESISTANCE TO ANTIBIOTICS

The use of antibiotics is limited because bacteria have evolved defenses against certain antibiotics. One of the main mechanisms of defense is inactivation of the antibiotic. This is the usual defense against penicillins and chloramphenicol, among others. Another form of defense involves a mutation that changes the bacterial enzyme affected by the drug in such a way that the antibiotic can no longer inhibit it. This is the main mechanism of resistance to the compounds that inhibit protein synthesis, such as the tetracyclines *(Holmberg et al., 1984)*.

All these forms of resistance are transmitted genetically by the bacterium to its progeny. Genes that carry resistance can also be transmitted from one bacterium to another by means of plasmids, chromosomal fragments that contain only a few genes, including the resistance gene. Some bacteria conjugate with others of the same species, forming temporary links during which the plasmids are passed from one to another. If two plasmids carrying resistance genes to different antibiotics are transferred to the same bacterium, their resistance genes can be assembled into a single plasmid. The combined resistances can then be transmitted to another bacterium, where they may be combined with yet another type of resistance. In this way, plasmids are generated that carry resistance to several different classes of antibiotic. In addition, plasmids have evolved that can be transmitted from one species of bacteria to another, and these can transfer multiple antibiotic resistance between very dissimilar species of bacteria *(Holmberg et al., 1984; Bedford, 2000)*. The problem of resistance has been exacerbated by the use of antibiotics as prophylactics intended to prevent infection before it occurs.

1.2 OBJECTIVES

1.2.1 OVERALL OBJECTIVE

To compare the effectiveness of bioactive compounds of dry, powdered plant parts of *F. sycomorus* and *P. thonningii* as antibiotic and vaccine substitutes on broiler performance, when used as feed additives.

1.2.2 SPECIFIC OBJECTIVES

1. To compare the proximate composition and Calcium and Phosphorus content of feeds to which selected plant parts of *F. sycomorus* and *P. thonningii* have been added
2. To determine the effect of the formulated feed rations with *F.sycomorus* and *P. thonningii* additives on the growth, feed consumption, feed conversion ratio and mortality of broilers.

CHAPTER 2

2.0 LITERATURE REVIEW

There is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues *(Hamm, 2002)*.Scientists are as well concerned that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria. Some consumer groups are avoiding meat from birds fed with diets containing antibiotics, especially in some countries that import 12 to 14% of the Brazilian broiler meat *(Bedford, 2000)*. Nevertheless, according to the United States Department of Agriculture (USDA), 100% of the broilers and turkeys, 90% of the swine and 60% of the beef cattle produced in the USA are fed antibiotics in the diet as growth promoters during the rearing period. In Brazil, with the exception "caipira" (meaning naturally reared) birds, probably almost all broilers are given growth promoters as additives in ration *(Hamm, 2002)*.

In the 1950s, the USA FDA approved the use of sub therapeutic levels of antibiotics in animal feeds. Antibiotics are fed to animals to prevent disease and promote growth (*NARMS, 2005*). However, the use of antibiotics in animal feeds has been linked to antibiotic-resistant bacteria *(Holmberg, et al., 1984)*. Consequently, many countries have banned the use of sub therapeutic levels of antibiotics in production animal rations. The United Kingdom banned the use of penicillin and tetracycline for growth promotion in the 1970s, the United States banned the use of Enrofloxacin in 1996 while Sweden and Denmark banned all growth-promoting antibiotics in 1986 and 1999, respectively *(FDA, 2000)*.

Since 2000, when the European Union completely banned the use and even possession on the farm of antibiotic growth promoters in animal feeding especially poultry, many approaches have been attempted to control or prevent subclinical diseases and to maximize growth performance and economic viability. Different medicinal plants (compounds) i.e., herbs, organic acids and essential oils seem to be candidates of interest as alternatives to antibiotic growth promoters (*Hernández et al., 2004*).

2.1 TRADITIONAL MEDICINAL PLANTS IN POULTRY AND/OR FARM ANIMALS

Due to growing concerns over antibiotic resistance, the use of antibiotics as growth promoters in animal diet could be limited due to public or regulatory pressures. Thus, current research involving feed additives for diets of young animals is focused on searching for alternatives to antibiotics that would have at least similar growth promoting effects of antibiotics without causing bacterial resistance. Phytobiotics is a term used to describe plant derived natural bioactive compounds, which affect animal growth and health, and is often applied to essential oils botanicals, and extracts derived from herbal plants. Some phytobiotics are known to have antimicrobial or antiviral activities *(Burkill, 1984).*

Medicinal plants play an important role in individuals and communities health. The medicinal value of these plants depends on some chemical compounds that produce a definite physiological action in the human body. The most important of these bioactive constituents of plants are phenolic compounds (resorcinol), saponins, alkaloids, flavonoids and Terpenoids *(Hill, 1952).* The state of medicinal plants research has been emphasized in many developing countries (*Edeoga et. al., 2005*). The awareness of the role of medicinal plants in health care delivery of developing countries has resulted in researches into traditional medicine, with a view to integrating it with modern orthodox medicine (*Sofowara, 1993*).

2.1.1 FOOD VALUE AND BIOACTIVE COMPOUNDS OF *PILIOSTIGMA THONNINGII*

Piliostigma thonningii is a leguminous plant belonging to the family *Caesalpinaceae*, a family that comprises of trees, shrubs or very rarely scramblers. The tree is perennial in nature and its petals are white to pinkish in color produced between November and April. While the fruit, which is a hairy, hard, flattish pod is produced between June and September. It turns rusty brown, woody and twists before splitting at ripening and usually persists on the tree *(Lock and Simpson, 1999)*.

Locally (in Zambia), *Piliostigma thonningii* whose common name is Monkey bread is a shrub or tree up to 6m high whose edible parts are pods, leaves and undeveloped flowers. Its food value extends to its organic acid content. It emerges frequently in the Chipya and savannah woodland when fields are left to fallow, with its pods mostly consumed between

August and November. It competes very little with maize in the field and it fixes nitrogen, hence improves soil fertili y (*Banda-Nyirenda et al., 2006*).

In other parts of the world the seed is called Abefe in the Yoruba land (Nigeria). Vernacular names for the fruit includ :, Musekese (Bemba), Camel's foot, Kalgo (Hausa) and Okpoatu (Ibo). *P. thonningii* gro vs in open woodland and savannah regions that are moist and wooded grassland in low ɔ medium altitudes. It is widely distributed in Africa and Asia. It is found growing abunda ıtly as a wild uncultivated tree in many parts of Nigeria such as Zaria, Bauchi, Ilorin, ?lateau, Lagos and Abeokuta (*Jimoh and Oladiji, 2005*).
The seeds of *P. thonning*ː fruits have been reported to be eaten by African antelopes and elephants while farmers iı the lower Savanna region grind up the seed as fodder for cattle during winter months (*Jin oh and Oladiji, 2005*). Different parts of *P. thonningii* have also been described as useful n edicinally. Its root and twig have been used for the treatment of dysentery, fever, infectic ıs, respiratory ailments, snake bites, hookworm and skin diseases (*Banda-Nyirenda ·t al., 2006*).
Jimoh, and Oladiji, (2005), carried out a qualitative determination of chemical and nutritional composition of *Piliostigm ı thonningii*. Pods of *P. thonningii* were found to be rich in carbohydrate and mineral (lements as shown in Table 2.1. Mineral analysis of *P. thonningii* using mineral contents of s ımples of defatted flour digested by concentrated nitric acid and per chloric acid (1:1v/v) Sodium (Na), calcium (Ca) and potassium (K) were estimated using emission flame pho ometer, with magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn) and copper(Cu) det ·rmined using atomic absorption spectrophotometry showed that both the pods and leav(s are good sources of antioxidant micronutrients such as iron, calcium, selenium (Se), zi ıc and manganese as shown in Table 2.2.

Table 2.1 Proximate comı osition of *Piliostigma thonningii* fruit

Component	Value (% composition)
Moisture content	6.71 ± 0)4
Ash	3.50 ± (04
Crude protein	0.5 ± 0.: l
Crude fibre	35.03 ±(11
Lipid	1.42 ± 0)3
Carbohydrates	23.00 ± (.24

Table 2.2 Mineral composition of *Piliostigma thonningii* fruit and seed

Minerals	Concentration (ppm)
Fe	781.70 ±232.90
Se	3.3 ± 0.[illegible]0
Ca	43.11 ± 0.34
Zn	0.016 ± 0.06
Mn	1.00 ± 0.02

According to *Jimoh* and *Oladiji (2005)*, phytochemical screening of the bark and pods for the presence of tannins, phenolics, glycosides, saponins, flavonoids, steroids, phylobatannins and triterpenes detected by the method described by *Odebiyi* and *Sofowara (1978)* showed the presence of saponins, flavonoids, phenolics, glycosides, anthraquinones as well as cardiac glycosides while tannins, steroids, phylobatannins and triterpenes were absent. Although the oil has a very low peroxide value and high iodine value, the physicochemical characteristics of the oil determined according to *AOCS (1973)* showed that it cannot be recommended for consumption because of the low yield as well as its repulsive odor. The pods, however, can serve as a cheap source of protein, energy, as well as antioxidant micronutrient supplement in both man and animal.

Table 2.3 Bioactive Compound Content of the *P. thonningii*

Chemical component	Described Function in animals *(Edward, 2005)*	Pods/ Fruit	Root-bark	Bark
Steroids	Physiological activity.	-	+	-
Terpenoids	Aromatic qualities, antibacterial, antineoplastic and pharmaceutical	-	+	-
Flavonoids	Antioxidant, antihistamine and antimicrobial	+	+	+
Tannins	Enhance growth performance	-	+	-
Saponins	Hemolytic, anti-inflammatory, immune-stimulating activity and antimicrobial.	+	+	+
Glycosides	Broken down into sugars by enzymes.	+	-	+
Anthraquinones	Control pigmentation and antioxidant	+	-	+
Alkaloids	Medicinal and Pharmacological use	-	+	-

Key + (Positive) = present; - (negative) = absent.

Source: Madara et al., 2012 ethanolic root-bark extract and **Jimoh and Oladiji, 2005** bark and pods extract

A research by *Omeke et al., (2001)* to investigate the effects of graded levels of ethanolic *Piliostigma thonningii* bark extract on feed conversion efficiency, consumption and daily weight gain on broilers fed diets containing 0.5, 1.0 and 2.0mg of the extract per kg feed respectively, with Control group receiving normal diet, showed the relatively promoted feed conversion efficiency and broiler growth rate particularly at its threshold level of 1.0 mg/kg feed with the optimum effect of the extract occurring by the 5th week and feed intake correlated positively with water intake.

2.1.2 BIOACTIVE COMPOUNDS OF *FICUS SYCOMORUS* AND THEIR EFFECTS

Ficus sycomorus Linn belongs to *Moraceae*, a family that is reputable for its medicinal values and consists of about 40 genera and over 1,400 species of trees, shrubs, vine and herbs, often with milky latex juices *(Zerega et al., 2005).*

In most parts of Zambia, *F. sycomorus* (common fig) is a large semi-deciduous tree that grows up to the height of 20 meters with widely spreading branches and a massive crown. *Ficus sycomorus* (common fig) whose edible plant plants are ripe fruits, leaves and the bark are available throughout the year in all woodlands of Zambia with the tree producing fruits two or more times a year (*Banda-Nyirenda et al., 2006*). The most edible parts are its fruit and leaves while its nutritive value lies in its content of protein, Vitamin A and B, sugars, iron, calcium, phosphorus and roughage (*Banda-Nyirenda et al., 2006).* According to *Banda-Nyirenda et al. (2006)* Sheep and cattle eat its foliage. *F. sycomorus* have been suspected to possess antidiarrheal properties and has been reported to be a potent antimicrobial agent.

Ficus sycomorus has many traditional medicinal uses in the treatment of snake bites, jaundice, chest pains, dysentery, cold, coughs and throat infections *(Sofowara, 1993)*. The mechanisms of action of the constituents of the organic fractions of the plant could probably be by already known mechanisms such as inhibition of electron transport chain, sphingolipid biosynthesis and fungal cell wall development *(Sofowara, 1993),* or yet some other undiscovered mechanisms.

Qualitative analysis of the phytochemicals of an aqueous extract of *Ficus sycomorus* from the leaves, stem-bark and root-bark of screened for chemical constituents by *Zaku, et al., (2009)* using standard procedures as described by *Sofowara (1993), Evans* and *Trease (1989)* and *Harborne (1973)* identified the bioactive (chemical) constituents as shown in Table 2.4 below.

Table 2.4 Bioactive Compound Content of the *F. sycomorus*

Chemical component	Described Function in animals (Edward, 2005)	Leaves	Stem-bark	Root-bark
Steroids	Physiological activity.	+	+	+
Terpenoids	Aromatic qualities, antibacterial, antineoplastic and pharmaceutical	+	+	+
Flavonoids	Antioxidant, antihistamine and antimicrobial properties	+	-	+
Tannins	Enhance growth performance	+	+	+
Saponins	Hemolytic, anti-inflammatory, immune-stimulating activity and antimicrobial.	+	-	+
Reducing sugars	Energy boosting	+	-	+
Anthracyanosides	Control pigmentation and antioxidant properties.	+	+	+
Alkaloids	Pharmacological effects and are used as medications	+	+	+

Key + (Positive) = present; - (negative) = absent.

Source: Zaku, et al., 2009.

CHAPTER 3

3.0 MATERIALS ANI METHOD

3.1EXPERIMENTAL MATERIALS

3.1.1 Collection of Pl nt Materials

The edible plant parts ol *Ficus sycomorus* and *Piliostigma thonningii* were collected from the naturally growing trees , round the University of Zambia Great East Road Campus.

The Bark of both tree s ecies was collected from the part of the trunk not facing the sun to avoid loss of water thr ugh transpiration and hence facilitate quicker recovery of the tree. The green leaves of bo h tree species were obtained from the already grown branches (i.e. mature branches) so as not to disturb the photosynthetic processes of the tree and hence conserve the tree specie . The green unripe fruit of the tree species were also collected.

3.1.2 Preparation of plant materials to be used in the feed rations

The plant materials req ired in the research were cut into small pieces and subjected to air drying (i.e. in the ab ence of direct sunlight) to prevent the loss of some bioactive compounds. The plant parts selected for the broiler feeding trials were the bark and leaves for both tree species an l only the fruit for *P. thonningii* because the fruit for *F. sycomorus* developed molds.

The air- dried bark for l oth tree species and fruit for *P. thonningii* were then ground using a hammer mill with a 3 m sieve. The leaves for both tree species were pounded using a mortar and pestle to a oid the loss of bioactive compounds due to heat produced by the grinding mill. The wh le mill and sieve were cleaned thoroughly before use and after grinding one sample to avoid cross- contamination. Different mortars and pestles were used for the leaves of the twc plant species respectively, for the same reason.

The powdered plant pa ts were mixed thoroughly before being used in the compounding of the treatment feed ratic ns. A 1% inclusion level in all the treatment rations was used as shown in Table 3.1. All the feed administered to the birds for the entire experimental period of six (6) weeks had the powdered plant additives except for the birds under conventional management, labeled as control units.

3.2 BROILER TRIALS

The broiler trial was carried out at the University of Zambia Field station (School of Agricultural Sciences, Department of Animal Science). 407 Cobb 500 day-old broiler chicks from Hybrid Poultry in Lusaka were used.

3.2.1 Treatments

Three (3) replications and six (6) treatments of:

1. *F. sycomorus* leaf,
2. *F.sycomorus* bark,
3. *P. thonningii* leaf,
4. *P. thonningii* bark,
5. *P. thonningii* fruit and
6. Conventional management.

3.2.2 Experimental Design

The trials were carried out in a Randomized Complete Block Design (RCBD), as shown in Table 3.1, with the blocking factors being:

1. Movement of people and animals.

   Birds in Block 1 and were expected to be the most affected due to the frequent movements near their wall.

2. Ventilation.

   The broiler trials were conducted during the rainy season when litter availability is a problem. Birds in Blocks 1 and 3 were expected to be better ventilated than the birds in block 2, because of their closeness to the windows of the poultry house. This would impact on the litter, as humid conditions would promote faster decomposition of the manure in the litter, and hence predispose birds to stressful conditions. The supply of cool fresh air to Block 2 would also be less. This would further increase the heat stress, in times that the temperatures in the poultry house were high.

3. Direct rays of the sun.

Birds in Block 1and 3 were expected to be affected by direct sunlight and heat in the morning and late afternoon, respectively, while the birds in Block 2 which was mid-way between 1 and 3 were not. The heat stress experienced by the birds in those two blocks was anticipated to slow the growth rates of the chickens in the affected blocks.

Table 3.1 Experimental Layout

BLOCK 1	BLOCK 2	BLOCK 3
P. thonningii leaf	*F. sycomorus* bark	Conventional mgt
F. sycomorus leaf.	*P. thonningii* fruit	*P. thonningii* leaf
F. sycomorus bark	*P. thonningii* bark	*F. sycomorus* leaf
P. thonningii bark	Conventional mgt	*F. sycomorus* bark
P. thonningii fruit	*F. sycomorus* leaf	*P. thonningii* bark
Conventional management	*P. thonningii* leaf	*P. thonningii* fruit

Eighteen (18) experimental units were used of which the six units in the first block (Block 1) had twenty three (23) chicks while the six experimental units in the last block (Block 3) had 22 chicks. The middle block (Block 2) had a varied number of chicks of between 22 and 23. The treatments were as shown in Table 3.2.

Table 3.2 Treatments and Dietary supplement Inclusion levels

Treatment	Dietary supplement	Dietary supplement Inclusion level in 100Kg: Starter Feed	Finisher Feed
1	Pounded *F. sycomorus* leaf	1% (1Kg)	1% (1Kg)
2	Ground *F. sycomorus* bark	1% (1Kg)	1% (1Kg)
3	Pounded *P. thonningii* leaf	1% (1Kg)	1% (1Kg)
4	Ground *P. thonningii* bark	1% (1Kg)	1% (1Kg)
5	Ground *P. thonningii* fruit	1% (1Kg)	1% (1Kg)
6	Conventional feed	0% (0Kg)	0% (0Kg)

The birds under Conventional management were fed conventional feed with 0% dietary supplement and vaccinated with Gumboro and Lasota while the rest of the birds were not vaccinated.

3.2.3 Broiler Management

3.2.3.1 Housing Preparation and Equipment

The poultry house that was used for the trials had a North-South orientation. It was disinfected using Vet fluid first and three weeks before the arrival of birds the house was sprayed using formalin.

The 21 experimental unit pens each measuring 2x1m for 20 birds were covered with polyethylene black plastic for heat conservation. The floor of the pens was covered with wood shavings. Infrared lamps placed in each pen were used for brooding. Before the chicks arrived the place was warmed to raise the temperature for the drinking water which was left in the house overnight.

3.2.3.2 Feed and Water Management

Vitamin and mineral supplements were given to the birds in water from the first day to the fifth week. The birds were fed using the two phase feeding system. The feed given was compounded as starter and finisher feeds (Table 3.3). The starter was given up to four weeks and the finisher was fed in the last two weeks of the experiment. Throughout the experimental period all the birds were given the feed *ad libitum*.

3.2.3.2.1 Feeds used in the experiment

A two phase feeding regime of compounded Broiler Starter at 23% CP and Finisher at 18% CP was used. The proportions and ingredients used in treatment feeds are as shown in Table 3.3

Table 3.3: Proportions and Ingredients used in the Feed Formulations

INGREDIENT	STARTER (kg)	FNISHER (kg)
No. 3 Maize Meal	49.38	65.18
Soy bean Meal	45.5	29.6
DCP	2.5	2.33
Limestone	0.8	1.0
Methionine	0.12	0.19
Salt	0.3	0.3
Broiler premix	0.4	0.4
Dietary Supplement	1.0	1.0
Total	100.00	100.00

3.2.3.3 General Bird Management

The chicks were collected from the Chamba Valley Hybrid Poultry Sales Offices. Upon collection and before transporting them to the University of Zambia Animal Science Field Station where the experiment was set up, the chicks were sorted and checked for any deformities. Upon arrival in the experimental poultry house the birds were counted, weighed and one by one their beaks dipped in the recently prepared mineral and vitamin supplement (stress pac) and then randomly placed in each experimental unit. The brooding period lasted only three weeks because environmental temperatures were high. Mineral and vitamin supplements in water were provided for four weeks there after 'plain' clean tap water was given. Feed was given *ad libitum* every morning throughout the broiler trial period.

3.2.3.4 Vaccination Programme

Only the birds on the Conventional management were vaccinated against Newcastle Disease (Lasota vaccine) and Infectious Bursal Disease. These birds were vaccinated first against Gumboro (Infectious Bursal Disease) on the 11th day of rearing and the second Gumboro vaccinated on the 21st day of rearing. On the 15th day and 25th day the birds were then vaccinated with the Lasota l lewcastle Disease vaccine.

3.3 DATA COLLECTI D

3.3.1 Weekly Live weight

The live weights of the birc s in each experimental unit were measured and recorded in the data collection sheets on the lay the chicks arrived. Thereafter the live weights of the birds in each experimental unit were aken weekly throughout the experimental period.

3.3.2 Feed Consumption

The amounts of feed given added to the feeders) to the birds were weighed and recorded daily.

3.3.3 Bird mortalities

The number of deaths for eac ı treatment during the trial period was recorded.

3.3.4 Proximate Analysis

The six **treatment** feed forı ıulations for both broiler starter and broiler finisher were analyzed for Moisture conte ıt, Mineral content (Calcium and Phosphorus), Crude Protein and Ether Extractives (Crude ›il).

3.3.4.1 Preparation of Samp es

The samples were prepared a cording to the stipulations of the methods used in the Weende Scheme. Two replications foı each experiment were done, with the average obtained as the content in the ration.

3.3.4.2 Methods Used

The Proximate Analysis wa: done using the AOAC methods (1998). Determination of Moisture content (using the l oss on ignition method) and the Extraction of Minerals was done as modified in the Univ rsity of Zambia, School of Agriculture Sciences, Department of Animal Science, Animal Nı trition Laboratory Manual.

Kjeldahl method determ ned the Crude Protein content while the Weibull method was used to determine the Crude Oil content The Permanganometric method was used to determine the Calcium content of the feeds, while Phosphorus was done using the Vanodo-molybdate method spectrophotometrically.

3.4 STATISTICAL ANALYSIS

The data on total live weight, feed consumption and FCR was sorted and trends produced using Microsoft Excel 2010.

Least Significant differences were used for mean separation with the analysis of variance obtained using Genstat 1 lth Edition Statistical package.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 PROXIMATE COMPOSITION

Even though the initial calculations of Crude Protein in all the starter rations was balanced at 23% CP the CP% in the ration that contained the *F. sycomorus* was the lowest among all the starter rations as shown in Table 4.1 but not so far from the recommended 22%CP in the Zambia Bureau of Standards (ZABS) for Broiler Feeds, and thus this did not affect the growth parameters as seen from the Analysis of variance.

Table 4.1 Proximate Composition of the Broiler Starter and Finisher

Feed Sample	Analysis						
STARTER	**M (%)**	**DM**	**Ash**	**Ca (%)**	**P (%)**	**CP (%)**	**EE**
F. sycomorus leaf,	9.73	90.27	10.42	1.63	0.49	21.55	6.22
F. sycomorus bark	9.41	90.59	8.31	1.21	0.30	23.75	8.06
P. thonningii leaf	9.92	90.09	8.17	1.74	0.35	23.31	6.50
P. thonningii bark,	9.60	90.40	8.18	1.62	0.31	23.00	8.20
P. thonningii fruit	8.55	91.45	7.36	1.36	0.21	22.99	8.52
Conventional management	8.92	91.08	8.61	1.62	0.29	23.01	5.68
FINISHER							
F. sycomorus leaf,	9.84	90.17	10.49	1.72	0.35	17.28	6.56
F.sycomorus bark,	10.17	89.83	9.66	2.25	0.40	20.11	6.40
P. thonningii leaf,	9.62	90.39	7.21	1.78	0.29	18.39	5.52
P. thonningii bark,	9.30	89.96	8.01	1.49	0.50	18.48	9.08
P. thonningii fruit	10.17	89.83	7.14	1.67	0.51	18.49	8.38
Conventional management	9.86	90.14	7.54	1.48	0.40	18.11	6.52

4.2 BIRD PERFORMANCE

The effect of the bioactive compounds in the edible plant parts of *F. sycomorus* and *P. thonningii* on the live weight, feed consumption and feed conversion ratio measured at 43 days is as shown in Table 4.2

Table 4.2 Bird Performance

Treatments	Mean LW (Kg)	Mean FC (Kg)	Mean FCR
F.sycomorus leaf,	2.616^{g}	3.717^{k}	1.440^{m}
F.sycomorus bark,	2.699^{g}	3.726^{k}	1.380^{m}
P.thonningii leaf,	2.668^{g}	3.767^{k}	1.410^{m}
P.thonningii bark,	2.716^{g}	3.850^{k}	1.420^{m}
P.thonningii fruit	2.740^{g}	3.946^{k}	1.440^{m}
Conventional management	2.599^{g}	3.717^{k}	1.377^{m}
Grand Mean	2.673	3.794	1.411
LSD (5%)	0.2532	0.269	0.0587
C.V %	5.20	3.90	2.29

Means in the same column followed by the same superscript are none significantly different from each other (P<0.05).

Key: LW = Live Weight; **FC** = Feed Consumption; **FCR**= Feed Conversion Ratio

4.2.1 Live Weight at 43 Days

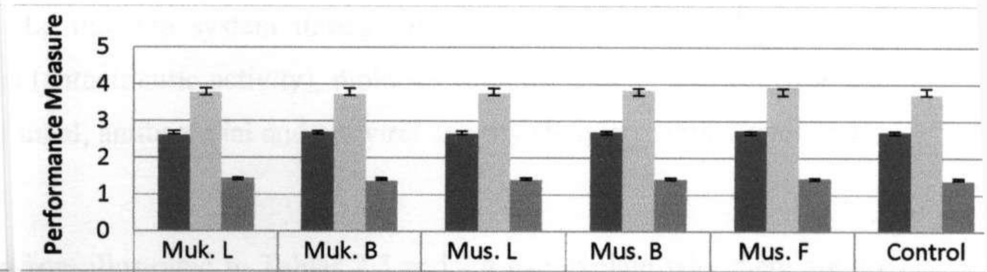
There were no significant differences in the mean live weight among the treatments at 95% confidence level *(P< 0.05)*

4.2.2 Feed Consumption at 43 days

There were no significant differences in the mean feed consumption among the treatments at *(P < 0.05)*.

4.2.3 Feed Conversion Ratio at 43 days

There were no significant differences in the mean feed conversion ratio among the treatments at both *(P < 0.05)*.



	Muk. L	Muk. B	Mus. L	Mus. B	Mus. F	Control
Average Live Weight (Kg)	2.617	2.695	2.66	2.705	2.738	2.701
Mean Feed Consumption (Kg)	3.767	3.723	3.75	3.84	3.944	3.716
Mean Feed Conversion Ratio	1.44	1.38	1.41	1.42	1.44	1.38

Fig 4.1 Comparison of Bird performance among treatments

4.3 EFFECT OF BIOACTIVE COMPOUNDS IN PLANT SPECIES ON GROWTH PARAMETERS

Antioxidants are capable of prohibiting oxidative processes while supporting disease prevention *(Rathgeber et al, 2008)*. Therefore, supplementation of synthetic antioxidants such as alpha-tocopheryl acetate or butylated hydroxytoluene to mitigate the oxidative stress has become a common practice in the poultry industry. Recently, use of plant extracts as natural antioxidants has gained increasing interest because of the global trend of restriction in use of synthetic substances especially with the demonstrated fact that antioxidant-rich plant extracts have potential benefits in treating coccidial infections *(Rathgeber et al, 2008)*.

Examples of antioxidants which are found in either or both of the plant parts of the *F. sycomorus* and *P. thonningii* in different concentrations include Flavonoids, Tocopherols, β-carotenoids, Vitamin C, Phenols and Selenium *(Rathgeber et al, 2008; Jimoh* and *Oladiji, 2005*). A reported by *Oladiji (2005*) showed that polyphenols are the most abundant antioxidants in plant parts of *P. thonningii* especially the bark and leaves and exhibit significant antiseptic and antimicrobial activity against bacteria, fungi, viruses and protozoa in a wide variety of food systems and environments.

Flavonoids like phenols are a diverse group of biochemical compounds known for their antioxidant potency but the specific effects attributed to flavonoids are anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral (*Jimoh* and *Oladiji, 2005*).

Saponnins can impact the immune system through their toxicity to insects (insecticide activity), parasite worms (anthelmintic activity), molluscs (molluscicidal), and fish (piscidal activity) as well as antifungal antibacterial and antiviral activity (Seigler, 1998; *Jimol et al., 2005*).

These and other effects from illustrated in Tables 2.3 and 2.4 can explain why there are no significant differences in the performance of broilers under the different treatments and those on conversional management even without vaccines.

4.4 BIRD MORTALITIES

A total of 10 birds died during the experimental period. The mortalities were all from the experimental units in Block 2 because of the heat stress that affected only the birds in this Block during the early days of the broiler trials. The lack of mortalities in the conventional management and *F. sycomorus* Bark units is attributed to the fact that their Block 2 units were at the two ends of the block, where there was more ventilation and hence better temperatures for the chicks.

Table 4.3 Total Bird Mortalities per Treatment

Treatment	**Total birds on day 1**	**Mortalities per Treatment**	**Percent Mortality**
F. sycomorus leaf,	68	2	2.94
F.sycomorus bark	68	2	2.94
P. thonningii leaf	67	0	0.00
P. thonningii bark	68	3	4.41
P. thonningii fruit	68	3	4.41
Conventional management	68	0	0.00
Total	407	10	2.46

CHAPTER 5

5.0 CONCLUSION

There were no significant differences in the live weight, feed consumption and the feed conversion ratio at 43 days among the experimental birds. This indicates the effectiveness of bioactive compounds of edible plant parts of *F. sycomorus* and *P. thonningii* as antibiotic and vaccine substitutes in broiler production.

It can be concluded that the bioactive compounds have an effect on the prevention of diseases when fed to broilers without affecting their performance. This is because none of the birds were observed to have suffered any infection even at times when the litter not dry (from day 40 to day 43). The comparable live weights, feed consumption and feed conversion ratios obtained at day 43 from the birds supplemented with edible plant parts and those on Conventional Management also indicates that bioactive compounds in these pounded or ground edible plant parts of *P. thonningii* and *F. sycomorus* had an immune enhancing effect on the broilers since the birds had normal growth even without vaccination against Newcastle or Infectious Bursal Disease during the rearing period. The highest mortality of only 3 birds in P. thonningii fruit and bark out of 68 birds is another indicator that the bioactive compounds in the edible plant parts have immune boosting factors and thus can substitute for antibiotics and vaccines.

The obtained results show that the bioactive compounds of edible plant parts of *F. sycomorus* and *P. thonningii* exerted effectiveness on performance of broilers. Therefore, it can be concluded that the dietary supplements have the potential to be applied as effective substitutes for in-feed antibiotics and oral vaccinations.

5.2 RECOMMENDATION

After the results obtained in this research, I recommend that the research is reconducted in different environments so as to be sure of the effectiveness of the edible plant parts on the performance of broilers.

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APPENDIX I: ANALYSIS OF VARIANCE

IA) Analysis of variance for mean live weight at 43 days

Source of Variation	Degrees of Freedom	Sum of squares	Mean sum of Squares	F calculated	F tabulated ($P < 0.05$)
Block	2	0.066	0.033	1.71	4.10
Treatment	5	0.047	0.009	0.47	3.33
Residual (Error)	10	0.194	0.019		
Total	17	0.307			

IB) Analysis of variance feed consumption at 43 days

Source of Variation	Degrees of Freedom	Sum of squares	Mean sum of Squares	F calculated	F tabulated ($P < 0.05$)
Block	2	0.085	0.043	1.95	4.10
Treatment	5	0.117	0.023	1.05	3.33
Residual (Error)	10	0.219	0.022		
Total	17	0.421			

IC) Analysis of variance for feed conversion ratio at 43 days

Source of Variation	Degrees of Freedom	Sum of squares	Mean sum of Squares	F calculated	F tabulated ($P < 0.05$)
Block	2	0.0008	0.0004	0.4000	4.10
Treatment	5	0.0117	0.0023	2.3000	3.33
Residual (Error)	10	0.0104	0.0010		
Total	17	0.0229			